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INTRANUCLEAR INCLUSIONS IN EXPERIMENTAL HERPETIC LESIONS OF RABBITS *

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Within cells of early lesions experimentally induced in rabbits with herpetic virus there occur characteristic intranuclear inclusions the presence of which, because of their constancy and their uniformity of structure and staining properties, serves to establish histologically the diagnosis of the herpetic nature of a given lesion in an animal inoculated with this virus. Similar intranuclear inclusions occur within epithelial cells of fresh vesicles of herpes simplex in the human.

In this respect the virus of herpes is similar to a large group of so-called filterable viruses which induce within cells of an infected tissue certain structures which are proper to the lesions characteristic of each. To this group belong the diseases smallpox, rabies, molluscum contagiosum, trachoma, Geflügelpocke, varicella and others. In most of these infections the corresponding "inclusions" are found within the cytoplasm of cells. The inclusions of herpes, however, occur only within nuclei. Lipschütz [1] and others have regarded the cellular inclusions of these various diseases as composed in part at least of the specific virus itself, and formed as a result of the proliferation of the infectious agent within the cell affected. While this hypothesis is a most useful one and has much to support it, further evidence is necessary to establish its truth. One cannot rely entirely upon the morphology of minute components of such structures to establish with certainty their parasitic nature. But whether or not

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it will eventually be proved that the inclusions and the virus are identical, nevertheless much can be learnt by clearly recognizing a constant association of a characteristic type of inclusion with any particular infection, and by identifying it with the lesion so that a diagnosis of the infection on this basis may be possible. In the study of such inclusions evidence for or against their hypothetical relation to the virus will from time to time accumulate.

Lipschütz,[2] Luger and Lauda,[3] and Lauda [4] have described so clearly the characteristics of the intranuclear inclusions of herpetic lesions, and our own investigations [5] with the virus of herpes simplex have so completely confirmed and extended their observations that it has been surprising to observe in certain recent publications a failure to recognize their uniform occurrence and an underestimation of the morphological importance of these bodies in herpetic lesions, aside from any consideration of their possible identity with the virus. Thus Cowdry and Nicholson [6] summarize the result of their study of various granulations in herpetic lesions: "It is our belief that the inclusions which are so abundant in herpetic lesions do not represent a concrete class of granulations *sui generis* but that they are of variable composition and are derived from several sources." They describe the nuclear inclusions of Lipschütz, but do not consider them any more specific for the herpetic lesion than various intracellular and extracellular granules obviously of degenerative origin. The issue has been obscured by the description of ill-defined, cytoplasmic and nuclear granules, by da Fano,[7] Levaditi [8] and others which either have nothing to do with the inclusions so clearly defined by Lipschütz or are so confused as to make impossible a judgment of their relation to them.

A clearer general recognition of the characteristics of herpetic intranuclear bodies is necessary to a more exact understanding of the nature of the virus, of the lesions it produces and of its behavior as an infectious agent.

Identification of these intranuclear structures as described by Lipschütz has been very valuable in investigations of local herpetic lesions [9] and of the manner in which the virus progresses from the periphery to infect the central nervous system, and it has seemed therefore advisable to restate the characteristics of these inclusions and to record our impressions as to their composition, structure and mode of formation.

The characteristic herpetic inclusion is confined strictly to the interior of the nucleus. We have found no type of granulation or other structure in the cytoplasm of cells or situated extracellularly which has any specific or constant relation to a herpetic infection.

The herpetic inclusions are to be distinguished from the preformed structures or products within the normal nucleus. They are distinct from the nucleoli and the products of nucleolar disintegration. Both morphologically and tinctorially it can be determined that the nucleoli play no morphological part in the formation of the herpetic inclusions. Nucleoli showing various stages of degeneration may be recognized in ganglion cells of the central nervous system in clear contrast to the herpetic bodies in hematoxylin-eosin preparations. The nucleoli may appear intensely red in preparations stained with carbol-fuchsin and counterstained with Loeffler's methylene blue and differentiated in alcohol. In such a preparation the herpetic bodies do not stain at all or may be faintly blue depending upon the degree of differentiation. The nucleoli stain intensely with acid fuchsin, in an acid fuchsin-methyl green preparation, while the herpetic bodies remain unstained or appear faintly green.

Nuclear chromatin is readily distinguishable from the material composing the inclusions by its avidity for basic dyes. In the disintegrating nucleus granules of chromatin still stain a deep blue after the eosin-methylene blue or Giemsa method.

The herpetic intranuclear inclusions are of a different structure and composition from the two nuclear constituents, chromatin and nucleolus. They are not to be distinguished in earliest stages, however, from material precipitated from the nucleoplasm by fixation in Helly's fluid. This is to be seen especially well in large motor ganglion cells of the central nervous system in which nucleoli are prominent and sharply circumscribed and nuclear chromatin is not abundant. In the interior of the nuclei of these cells fixed immediately after death by direct injection of Helly's fluid into the carotid arteries there are one or more groups of amorphous finely granular material, which seem to have precipitated about certain centers (Fig. 1). Elsewhere irregularly distributed within the nucleus there may be a similar material giving a somewhat reticulated appearance to the nucleus. This material has the same tinctorial properties as the herpetic inclusions, but differs from the typical, well-developed herpetic inclusion morphologically.

The herpetic bodies stain best in our experience with eosin or erythrosin and stand out in good contrast to particles of chromatin in combinations of these stains with methylene blue, hematoxylin or other basic dyes. In certain forms to be described they are amphophilic, taking a fairly sharp blue in eosin-methylene blue preparations stained deeply with the basic dye following fixation in Helly's or Zenker's fluid.

In the form in which the inclusions most commonly occur they are readily recognizable and are associated with other nuclear or cytoplasmic changes which accentuate their prominence.

It is in ganglion and neuroglia cells of the central nervous system that it has been possible to study the inclusions to best advantage.

In motor ganglion cells they are readily distinguishable from the coagulated nucleoplasm of normal cells by their size and configuration. The material of which they are constituted greatly increases in amount and may form a compact crescent or ring about the nucleolus, and eventually it completely fills the intranuclear space which coincidently enlarges. In ganglion cells there is not the same tendency for fluid to accumulate within the nucleus as in other types of cells in herpetic lesions, and in consequence the intranuclear body may not be separated from the nuclear membrane by a clear zone. The nucleolus shows evidences of disintegration when the inclusions are well developed. It loses its symmetrical contour, becomes vacuolated or breaks up into irregular granules. The chromatin is collected about the nuclear membrane. The cytoplasm of such a ganglion cell shows chromatolysis partial or complete (Fig. 2).

In other cells of the rabbits in tissues acutely infected with the virus of herpes simplex, an intranuclear material with staining properties identical with that of herpetic inclusions in ganglion cells presents an even more conspicuous structure.

Intranuclear inclusions of this description have been found within cells from all three germinal layers infected with the virus. The nucleus tends to enlarge, chromatin particles collect about the nuclear membrane and the "inclusion" occupies the center of the nuclear space frequently separated from the nuclear membrane by a clear zone. Well-developed inclusions are often single; in earlier stages, however, there may be several irregular particles which seem later to coalesce to form one structure. The outline of the mass conforms in general to the shape of the nucleus, and may be quite irregu-

lar, round, oval or elongated. The more compact the inclusion is the more intense is its coloration by eosin. Usually the masses are not perfectly homogeneous and hyaline in structure but appear more or less honey-combed or roughened.

These are the forms of the inclusions which one first observes and which serve to distinguish the herpetic nature of a given lesion. But it is to be noted that the herpetic bodies are not static structures; they are progressive in their development. They tend to increase in size and eventually to fill the nuclear space. When the included material completely fills the affected nucleus a change may frequently be noted both in its tinctorial property and in its structure. The included mass in this stage takes a faintly basic stain in preparations in which smaller inclusions are stained a clear red with eosin, and they no longer appear rough and irregular in composition but seem to be composed of very minute bodies closely arranged and uniform in size. At this stage irregular granules of chromatin are distinguishable by their deep and sharp basophilic stain, their irregularity in size and their peripheral arrangement. In most tissues it is impossible to resolve clearly the small bodies constituting the inclusion, partly because of their minute size and compact arrangement, and partly because of the difficulty in staining the individual particles intensely. Even in this stage, nevertheless, the masses are readily recognizable as herpetic inclusions.

We have found in rabbits that the clearest preparations of the granules constituting the herpetic inclusion are to be obtained by inoculating herpetic virus directly into a corpus luteum of early pregnancy. At the end of 24 hours the corpus luteum cells contain well-developed inclusions and the ovary is rich in virus as has been determined by inoculating ground ovarian tissue into the brain or on the cornea of rabbits. The interstitial and corpus luteum cells show in great numbers the discrete form of inclusions separated by a clear zone from the nuclear membrane. In many of the larger cells of the corpus luteum, however, the nuclei enlarge to several times their normal diameter and become filled with extremely minute round or oval granules of uniform size sufficiently separate to be fairly well resolved. Irregular and deeply staining granules of chromatin are readily distinguishable from them. The structures constituting the herpetic inclusion are difficult to stain. They are amphophilic but can be brought out fairly sharply in blue in eosin-

methylene blue preparations deeply stained with the basic dye and not differentiated very far with colophonium acetone, dehydrated with acetone and mounted in cedar oil, or colored by a modified Giemsa's stain as recommended by Wolbach [10] but rendered slightly more alkaline.

The bodies thus demonstrated are smaller, more uniform in size and more numerous than the similar structures depicted by Lipschütz in preparations stained by Weigert's method after fixation in sublimate alcohol (Fig. 3).

It is not purposed here to attempt to identify these minute structures with the virus of herpes simplex. They have been described as they were found, constituting a phase in the development of the intranuclear inclusion of experimental herpetic infections in rabbits. For various reasons discussed elsewhere the conclusion has been reached that the virus of herpes proliferates within cells, and we hold with Lipschütz that the intranuclear body is a manifestation of the presence of the virus within the nucleus. It seems evident, however, that the material which constitutes the "inclusion" may partially at least be composed of coagulated nucleoplasm which may impart the acidophilic staining property of the inclusions. It is to be noted, however, that when the minute granulations are discrete enough to be recognized as such they stain faintly basophilically, whereas the precipitate from the nucleoplasm of normal cells is more acidophilic. They are to be regarded at present as elementary bodies taking part in the structure of the herpetic inclusions.

The nucleoplasm according to recent cytological investigations contains no preformed structure other than the nucleolus,[11] yet the herpetic inclusions may be seen in fresh unfixed preparations.[12] Consequently if there is a precipitation other than that due to fixation, it must be a result of changes attendant upon the infection. The indications are that the "inclusions" are present within the nuclei before the death of the cell, for they may be demonstrated typically within cells of the cornea and skin whose nuclei have divided to form the multinucleated giant cells which Unna described as constituting in part the "ballooning degeneration."

It remains to be proved whether or not these uniform granules which enter into the composition of herpetic intranuclear inclusions represent the virus itself, but in this respect the problem is the same as that presented by certain of the "inclusions" of other diseases,

which in the cell appear amorphous, but are really composed of minute particles of uniform size having the morphological appearance of microörganisms. The acidophilic cytoplasmic bodies of Geflügelpocke offer a striking analogy.

At the present time it is of most importance to recognize in these nuclear inclusions a change which is brought about specifically by the virus of herpes simplex, or closely related viruses. This fact to the writer is clear and it is believed will be readily recognizable if proper precautions are observed, and if early lesions of herpes are submitted to histological investigation. With the strains of virus which we have used the herpetic bodies may be studied to best advantage in lesions produced at the end of 24 hours after inoculation, wherever the site of inoculation or infection may be. The time element is a most important one if uniform results are to be expected, because the inclusions disappear rapidly in lesions caused by this virus, and in direct proportion active virus diminishes.

In studying herpetic lesions of the central nervous system it is frequently found that the initial lesion present at the entrance of a particular nerve may show few or no cells containing herpetic inclusions, while later lesions, as for instance, at the base of the cerebrum, will show innumerable ganglion cells thus affected. For this reason we cannot agree entirely with Parker [13] that human encephalitis lethargica is distinguishable from an herpetic infection because of the absence in the lesions of herpetic intranuclear bodies. In the event of an herpetic encephalitis of the human, the lesions to contain inclusions, presumably, judging from our experience with rabbits, would have to be very acute, and one would not expect to find them in every lesion but only in those representing latest extensions of the infection.

Recognition of the fact that an infection with the virus of herpes simplex is accompanied by these characteristic intranuclear inclusions in cells of local lesions is of importance not only in aiding to establish the herpetic nature of a given experimental lesion, but their presence serves to correlate histologically the lesion of three human diseases, herpes simplex, herpes zoster and varicella. Similar intranuclear inclusions ocur in cells of the cutaneous eruption of each of these infections and they are not found so far as we know in any other human diseases.

Corresponding to the histological similarity of the cutaneous le-

sions of these three diseases evidence is accumulating that they may be caused by very similar viruses. It has been shown that an experimental disease can be produced in rabbits and guinea pigs with the virus of herpes simplex quite analogous in many of its features to human herpes zoster,[14] and clinically there is considerable evidence that herpes zoster and chicken pox are closely related infections.

A better understanding of the so-called filterable viruses will undoubtedly throw much light upon the nature of the cellular inclusions which accompany the lesions of many of the acute infectious diseases of this nature, and in the meantime careful study of the bodies may lead us to a truer conception of the nature of this class of infectious agents, and to a closer critical analysis of the pathology of a most important group of diseases.

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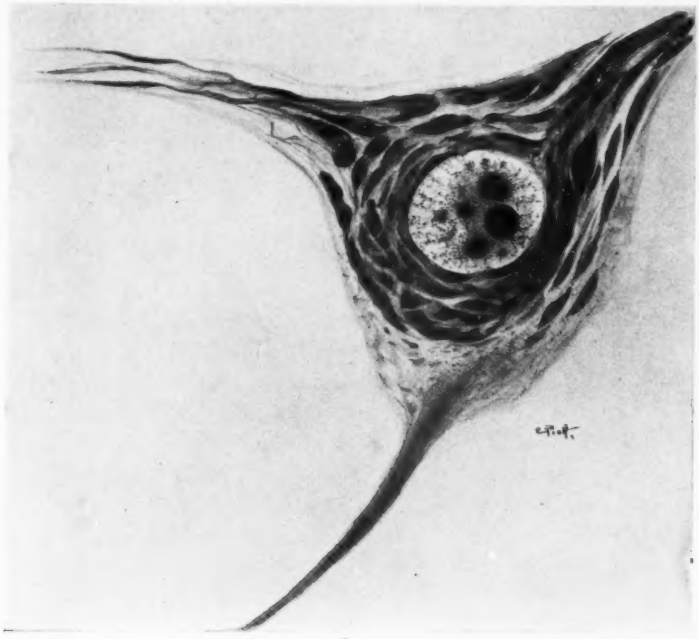
DESCRIPTION OF PLATE I

Drawings made from sections using No. 4 compensating ocular and 1.5 mm. objective.

Fig. 1. Normal multipolar motor ganglion cell from the motor nucleus of the fifth cranial nerve of a rabbit, showing dark circumscribed nucleolus about which are collections of material precipitated from the nucleoplasm. Nissl substance is abundant. Fixed in Helly's fluid, stained with hematoxylin and eosin.

Fig. 2. Multipolar ganglion cell from the motor nucleus of the fifth cranial nerve of a rabbit after inoculating the corresponding masseter muscle with virus of herpes simplex. The nucleus contains an irregular and vacuolated nucleolus partially surrounded by an irregular herpetic inclusion. Nissl substance has disappeared from the cytoplasm. Fixed in Helly's fluid. Stained with hematoxylin and eosin.

Fig. 3. Corpus luteum cell of a pregnant rabbit. The ovary was inoculated 24 hours before removal with virus of herpes simplex. The nucleus is enlarged and filled with minute structures uniform in size. The darker and somewhat larger bodies are particles of nuclear chromatin. There is an exudate of mononuclear leucocytes about the cell. Fixed in Helly's fluid. Stained with eosin and methylene blue.



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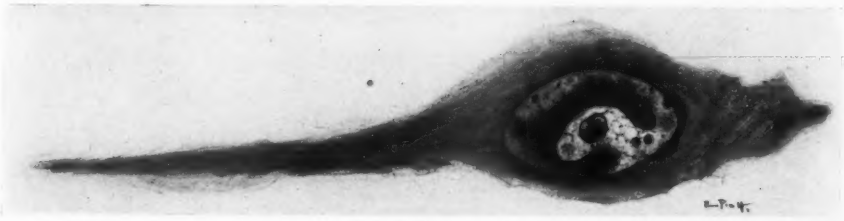

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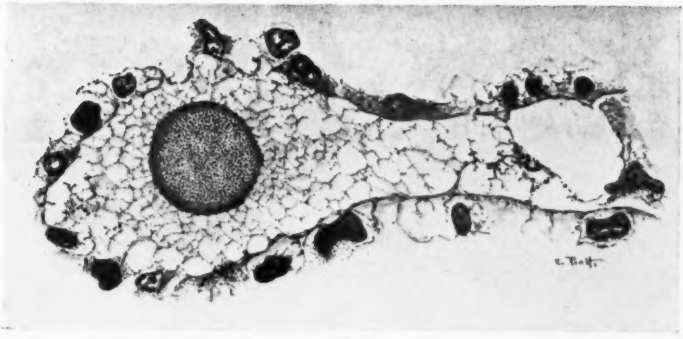

3

Goodpasture Herpetic lesions of rabbits

THE AXIS-CYLINDERS OF PERIPHERAL NERVES AS PORTALS OF ENTRY TO THE CENTRAL NERVOUS SYSTEM FOR THE VIRUS OF HERPES SIMPLEX IN EXPERIMENTALLY INFECTED RABBITS *

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A strongly neurotropic strain of the virus of herpes simplex derived from a herpetic vesicle on the lip of a patient with lobar pneumonia (virus M) has now been propagated through a series of rabbits by inoculations upon the scarified cornea for sixteen months and has, since its primary transfer to these animals, constantly induced a herpetic encephalitis when a uniform technique has been observed. With this strain it has been proved that the virus reaches the central nervous system through the medium of nerves supplying the peripheral areas primarily inoculated.[1, 2] This mode of entry of the virus from the inoculated cornea into the pons has been confirmed by the experiments of Marinesco and Draganesco.[4] It has appeared that an initial local infection of cells at the site of inoculation is necessary for such an extension of the virus along the nerves into the central nervous system.

At the central origin or terminus of a nerve thus conveying the virus there is produced an acute local herpetic lesion which has been demonstrated grossly and microscopically.[3] The lesion is a destructive one and is characteristic for this infection, the cells involved presenting the acidophilic intranuclear inclusions of Lipschütz which have been shown to be a histological criterion of a herpetic lesion.[5]

The virus may be conveyed along sensory, motor or sympathetic nerves depending upon the innervation of the peripheral site inoculated, as for example, following an infection of the cornea an acute herpetic encephalitis results involving the sensory root of the fifth cranial nerve in the pons and medulla on the side inoculated; following an injection of the virus into the muscles of a hind leg an acute myelitis is produced in the lumbar portion of the spinal cord; or if the virus is inoculated into an adrenal gland or an ovary an

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acute myelitis follows, striking the level of the spinal cord where sympathetic fibers from these organs enter. Inoculations into many other localities have given abundant confirmation of a neural transmission of the virus from a peripheral focus of infection and its ready transplantation upon the brain or spinal cord at its point of entrance where conditions for its growth and extension are exceptionally favorable.[2]

In a previous study of the neural transmission of the virus of herpes from a peripheral focus of infection to the central nervous system the opinion was expressed that the virus passed along axis-cylinders rather than by way of perineural spaces. This conclusion was arrived at from a consideration of several points. The virus seemed to grow within cells rather than in the tissue fluids. We were unable to transmit a herpetic infection by injecting the blood of an infected rabbit into a normal susceptible rabbit, nor were we able to cause an encephalitis by inoculating the virus into loose connective tissue, care being taken to avoid an infection of the epidermis about the wound. The presence of acidophilic intranuclear inclusions of Lipschütz[6] was regarded as representing an intracellular growth of the virus. The lesions produced within the central nervous system following a peripheral infection were proved to be directly associated with the nerve from the periphery, and the fibers of this nerve evidently directed the course of an extending infection within the brain or spinal cord after the virus had gained an entrance. The axis-cylinders being protoplasmic processes of ganglion cells, it was thought probable that the virus actually invades and grows along them.[4] However, the animals in our previous experiments were permitted to live usually for several days after they had shown evidences of cerebral disease and on histological examination the herpetic lesions had extended considerably from the point of entrance into the brain and there was always an associated local meningitis at the point of entry, consequently it was felt that the possibility of a perineural dissemination of the virus could not be entirely excluded.

The following experiments, it is believed, present convincing evidence that the virus does extend along the axis-cylinders and not, as maintained by Marinesco and Draganesco, through perineural spaces, and that it invades the central nervous system from a peripheral focus of infection by propagating itself along these cellu-

lar processes which for the most part in their extracranial courses are protected from other tissues by their myelin sheaths and the sheaths of Schwann.

Satisfactory conditions for the experiments were found in the anatomical structure of the fifth cranial nerve, and the situation of its motor nucleus in the depths of the pons and separate from the large descending crescentic bundle of its sensory fibers.

The fifth motor nerve throughout most of its extent lies in close proximity with sensory fibers. After it passes through the Gasserian ganglion, which folds partially about it on either side from above, and branches downward, it is accompanied for a considerable distance by the inferior maxillary division of the nerve. Between sensory and motor fibers there are scarcely enough delicate fibrils of connective tissue to make the limits of each group distinguishable. The two types of fibers are readily distinguishable in microscopic sections, however, because the axis-cylinders of the motor nerve are larger and are surrounded by a more compact-appearing myelin sheath. This greater diameter of the motor nerve fiber is quite marked at the proximal end. There is also a greater number of neurolemma cells in the sensory portion of the nerve.

The sensory and motor divisions of the fifth nerve enter the brain together, the latter having a ventral position; and a short distance from the brain they both suddenly lose their sheaths of Schwann in a sharp transverse plane which is common to both. Central to this point the myelin sheaths appear much looser and less substantial than peripherally, and neuroglia cells are situated here and there between the fibers, most numerously in the sensory division, yet the motor nerve continues to have the appearance of being more substantially protected by myelin.

On entering the pons the motor and sensory divisions separate, the sensory group forming a crescentic tract which extends caudally near the periphery traversing the medulla, while the motor division enters the pons as a compact group of fibers, extends dorsally and medially, the major portion finally arching caudally to end in a circumscribed group of large multipolar motor ganglion cells in which they have their origin and which constitute the motor nucleus. A smaller group of fibers continues dorsally to end in the mesencephalic or accessory motor nucleus situated laterally at the angle of the floor of the fourth ventricle. In the proximal third of their in-

tracerebral course the myelin sheaths are more delicate, and they gradually disappear as the nerves penetrate the nucleus.

The presence of the myelin protective sheath is of much importance in interpreting the results of the following experiments. It has previously been shown that following an inoculation of the cornea on one side there may be no certain lesion of the extradural portion of the sensory division of the fifth cranial nerve which innervates the cornea and along which the virus passes to the brain, but there is constantly an acute herpetic lesion immediately central to the transverse plane where the sheath of Schwann disappears, and from there it may extend throughout the intracerebral course of the sensory tract. It was believed this sharp delimitation of the lesion was to be explained by the fact that in the proximal portion of the nerve the axis-cylinders are less well protected by a myelin sheath permitting the virus to escape from infected axis-cylinders and to enter the susceptible neuroglia cells which surround them. But in the case of a sensory nerve it was not possible to determine accurately whether the virus followed the axis-cylinder to its termination, or whether liberated into perineural spaces on its entrance into the cerebral tissue because of less protection by myelin sheaths it was only directed in its spread by the bundle of fibers in which it happened to be situated. The conditions were suspected to be different, however, with the motor division of the fifth cranial nerve, having its central origin in large ganglion cells deeply situated within cerebral tissue; for if, as was assumed, the virus traversed axis-cylinders then the motor ganglion cells should show evidences of infection very early, and since the myelin sheaths of the intracerebral portion of the fifth cranial nerve are apparently more substantial than those of the corresponding sensory division, it was believed that the chances of escape of virus along the intracerebral extent of the nerve would be less. If the virus passed merely along perineural spaces on the other hand, one would expect it to be liberated at the entrance of the nerve into the brain, giving rise to an infection of neuroglia cells at this point with an accompanying local meningitis and herpetic lesions perhaps throughout its extent, but naturally involving neuroglia along the nerve and in the nucleus before the ganglion cells were affected, the latter perhaps escaping altogether in early stages.

To test this hypothesis it was decided to select as a point of attack the motor division of the fifth cranial nerve by inoculating the virus

of herpes directly into the masseter muscle and killing the rabbit at the first elevation of temperature, which we believe is the earliest indication of a herpetic encephalitis. By means of serial sections through a proper region of the pons the complete intracranial course of this nerve could be studied and the progress of the infection observed.

By this procedure it has been possible to show that following inoculation of the masseter muscle on the right side, intracerebral herpetic lesions are first demonstrable within the motor nucleus of the fifth cranial nerve, and the motor ganglion cells are apparently the first to be attacked as was anticipated, the medullated fibers passing through a wide extent of susceptible tissue without evidences of neuroglia infection, which would hardly be possible were the virus outside the medullary sheaths. Under these circumstances, there may be no evidence of a neuritis in the extradural portions of the motor nerve.

Material for inoculating the masseter muscle was obtained by inoculating the cornea of a rabbit with 24 or 48 hour virus (from an infected cornea) and at the end of 24 hours, anesthetizing the animal, scraping the cornea lightly with a sterile scalpel, and taking up the material from the conjunctival sac by washing off the cornea with 1 or 2 c.c. of sterile salt solution and aspirating with a syringe. From one half to the entire amount of material thus removed, suspended in about 1 c.c. of salt solution, was used for a single inoculation. In inoculating the virus into the muscle the material has been injected into several places among the muscle fibers without removing the needle, but by changing its depth and direction. The animals were then killed by etherization and exsanguination at the first distinct elevation of rectal temperature.

The brain was immediately fixed by injecting Helly's fluid under considerable pressure from a syringe into each carotid artery. It was then removed and placed in the same fixing fluid for 24 hours. Blocks were cut from various portions including the entire brain in cross section. In no instance in the following experiments was a cerebral herpetic lesion found beyond the motor division of the fifth cranial nerve and its nucleus on the side inoculated, excepting in Experiment 7, in which the rabbit was permitted to die of the infection. Here the infection had extended throughout the medulla and to the base of the cerebrum. Consequently in most of the ex-

periments described below only sections including the fifth motor nerve and its central distribution are recorded.

The following are typical experiments in most of which serial sections were studied.

Experiment 1.

R. 148–24.

1/9/24. Adult rabbit. Injected 0.5 c.c. suspension of pus and scrapings from right eye of R. 147 (inoculated 36 hours previously) into multiple places in right masseter muscle.

1/11. Temp. 103° F.

1/13. Temp. 103°.

1/14. Temp. 105.8°. Etherized. Brain, right fifth nerve and right masseter muscle saved for section.

Microscopic Examination. Serial sections through the pons at the level of the fifth motor root and nuclei. Microscopic sections show no meningitis at the entrance of the nerve and no lesion along the course of the nerve to the motor nucleus. Within this nucleus on the right side there is in places a slight cellular infiltration with phagocytic mononuclear cells and here and there are neuroglia cell nuclei containing typical herpetic intranuclear bodies. The main lesion, however, is to be found in several motor ganglion cells. Some of these show typical intranuclear bodies, sometimes with nucleoli intact, and chromatolysis. Others are necrotic and shrunken. There is no neurophagocytosis. The motor nucleus on the opposite side is quite normal and there is no lesion in the pons other than the foci about ganglion cells within the fifth motor nucleus. The sensory bundle of the right fifth nerve shows no lesion. In one section after the motor bundle has reached the level of the motor nucleus and is turning inward toward it, two neuroglia cell nuclei are found between nerve fibers containing intranuclear inclusions. There is no other evidence of infection here. Similar cells are found in other sections, but always at the central end of the nerve.

Serial sections through the right fifth motor nerve including the entire portion from the dura through the Gasserian ganglion and beyond, a total extent of over 1 cm., show no lesion.

This case shows that the virus may be transmitted along the motor nerve without evidence of its presence until the terminal third of its intracerebral portion is reached, that is, on a level with the motor nucleus. Here the myelin sheath is thin and axis-cylinders are poorly protected so that virus within axis-cylinders may escape and infect neuroglia cells with which it comes in contact.

Experiment 2.

R. 133. Adult rabbit.

12/21/23. Right masseter muscle inoculated in several places with washings from right eye of R. 131 (24 hour virus).

12/24. Temp. 104° F. Etherized. Autopsy negative.

Pons at entrance of fifth cranial nerves. There is no cellular exudate in the meninges about the entrance of the fifth nerve on the right or elsewhere. The motor bundle can be followed throughout its intracerebral extent and shows no lesion nor the presence of any intranuclear bodies in neuroglia cells. In the

upper pole of the right motor nucleus, however, there is a moderate general infiltration with mononuclear phagocytes. In small foci they are collected about necrotic ganglion cells. Many neuroglia cells immediately about the dead cells contain typical intranuclear herpetic inclusions. No herpetic lesion was observed elsewhere in the brain.

Right fifth motor nerve. Serial sections through the right fifth nerve were made, including that portion from a point several millimeters distal to the Gasserian ganglion to a point central to the plane intradurally where the sheaths of Schwann disappear, a total distance of 1.5 cm. Throughout this entire extent no lesion of the nerve was observed and no intranuclear herpetic inclusions were found in the cells of the neurolemma.

In this case, therefore, the only herpetic lesion found in the distribution of the nerve was at its central termination in the motor nucleus, and here as has been uniformly the case ganglion cells as well as neuroglia were destroyed by the herpetic infection.

Experiment 3.

R. 224. Adult non-pregnant female rabbit.

3/21/24. Right masseter muscle injected with virus in saline washings from left eye of R. 219 (inoculated 24 hours previously).

3/23/24. Temp. 102.8° F.

3/24/24. Temp. 103°.

3/25/24. Temp. 103°.

3/26/24. Temp. 105.8°. There is edema and congestion of the right eye and lacrimation. No pus is present. Cornea is clear. No turning of the head. Etherized.

Microscopic Description.

Serial sections of the pons at the entrance of and including the central distribution of the motor division of the fifth cranial nerves. At the entrance of the right fifth cranial nerve and over the base of the pons there is a slight exudate of mononuclear cells in the meninges. No lesion of the motor nerve is observed until a point half way in its intracerebral course is reached. Here there is a small focus of mononuclear phagocytes, but no intranuclear bodies are found. Central to this point there are small foci of such cells but in inconspicuous numbers. No relatively significant herpetic lesion is found until the nerve enters the motor nucleus. In the right motor nucleus there is an abundant mononuclear phagocytic cell exudate throughout its entire area and many necrotic ganglion cells are found, some of them surrounded by a small group of these cells. An occasional polymorphonuclear leucocyte is observed. Notwithstanding the abundant cellular exudate, only an occasional neuroglia cell contains an intranuclear inclusion. The exudate is apparently due entirely to the destruction of motor ganglion cells. Some of these are completely destroyed, others show typical herpetic inclusions and are undergoing disintegration.

Right fifth cranial nerve. Serial sections were made through this nerve including the motor bundle from a point distal to the Gasserian ganglion to near its entrance into the brain, a length of approximately 1 cm. Throughout its course one finds here and there small groups of mononuclear phagocytic cells situated among the fibers. These foci are not numerous but are very definite.

Careful study of these lesions proves each of them to be an herpetic neuritis. Each focus of cells is collected about an individual motor fiber which can be

traced through many sections. The entire extent of the fiber is not involved, the exudate occurring only at certain points. Three or four fibers presenting a surrounding exudate may be found in a low power field including the entire breadth of the nerve, and there are a few mononuclear phagocytes about small blood vessels in the neighborhood of the lesion. The earliest changes noted in such lesions are in the nucleus of a cell of Schwann's sheath. The nuclear chromatin is granular and thin, lying peripherally along the nuclear membrane. The interior of the nucleus is almost filled by a homogeneous or finely granular eosin-staining material characteristic of the herpetic intranuclear inclusions found so frequently elsewhere in acute herpetic lesions. The axis-cylinder immediately beneath such a cell takes a deeper eosin stain than others and it appears homogeneous or very finely granular and vacuolated. In more advanced lesions there is an accumulation of amoeboid mononuclear phagocytes arranged about the individual nerve fiber, and these cells seem in places to have penetrated the myelin sheath and to be phagocyting particles of the axis-cylinder, as several were found in such a situation containing globules of pink staining hyaline material within their cytoplasm. The nerves have not been stained for fat and no certain changes in the myelin sheaths have been demonstrated.

The relation of the cells of the neurolemma which contain herpetic inclusions to the lesions is such as to leave little doubt that the cellular exudate occurs only about those fibers, and at those points along an individual fiber, where a cell of Schwann is infected with the virus of herpes.

Experiment 4.

R. 275. Adult rabbit.

4/26. Right masseter muscle injected with 1 c.c. of a salt solution suspension of material from both eyes of R. 274 (inoculated 24 hours previously).
4/29. Temp. 102.6° F.
4/30. Temp. 102.7°.
5/1. Temp. 102.5°.
5/2. Temp. 102.8°.
5/3. Temp. 104°. Etherized. Autopsy negative.

Microscopic examination of the brain. Paraffin blocks were made from various cross sections of the brain and spinal cord. Sections showed no evidence of herpes other than in the pons as described below. Serial sections were made through the intracerebral distribution of the fifth cranial nerves and motor nuclei. In the intradural portion of the motor root of the fifth nerve there are small foci of mononuclear cells here and there, but no intranuclear bodies are found. There is a sharp change at the point where the nerve loses its sheath of Schwann. Here there is an abundant exudate of mononuclear leucocytes and some polymorphonuclear leucocytes. Many cells are found in foci of exudate which contain typical herpetic intranuclear bodies. There is about the nerve here a cellular exudate in the meninges consisting of mononuclear leucocytes. A few neighboring blood vessels in the peripheral brain tissue are surrounded by a mantle of mononuclear cells.

The acute herpetic lesion of the nerve extends irregularly and with diminishing intensity from the line where the sheath of Schwann is lost until the nerve enters the brain. From this point no herpetic lesion is found in the nerve as it courses through the brain until the fifth motor nucleus is reached. In the nucleus and limited by its confines there is a general moderate infiltration with

mononuclear leucocytes, which in several small foci are grouped together. There is also a slight perivascular infiltration. Here and there neuroglia cells contain the herpetic inclusions in their nuclei, and necrotic ganglion cells are found showing chromatolysis and intranuclear herpetic inclusions. Grouped about such cells is a margin of mononuclear leucocytes.

This animal lived two days longer than the other acute cases after inoculating the masseter muscle, and there is an acute herpetic neuritis of the intradural portion of the motor nerve. Proximal to this point, however, the nerve presents no lesion, but there is an extensive circumscribed herpetic lesion of the fifth motor nucleus.

Experiment 5.

R. 276. Adult female.

4/26/24. Right masseter muscle inoculated by injecting salt solution suspension of material from both corneas of R. 274 (inoculated 24 hours previously).
4/29. Temp. 102.4° F.
4/30. Temp. 102.4°.
5/1. Temp. 104.5°. Etherized. Autopsy negative except for early pregnancy.

Microscopic Sections. Serial sections were made including the entire intracerebral extent of the motor root and nucleus of the fifth cranial nerve. In sections through the anterior pole of the nucleus there is a moderate infiltration with endothelial leucocytes, which in one or two places are grouped in small aggregations. There are no intranuclear bodies found here in the neuroglia cells. The cellular exudate is strictly limited to the nucleus and there are several degenerated ganglion cells, some of which show typical intranuclear bodies. The necrotic ganglion cells are the center of an accumulation of mononuclear leucocytes. There is slight perivascular infiltration in the neighborhood of necrotic ganglion cells.

In the terminal third of the motor root there is a small group of cells among which are one or two neuroglia cells containing herpetic inclusions. There is a slight increase in the number of mononuclear leucocytes in the meninges over the region of entrance of the fifth nerve, unassociated with any lesion of the nerve at this place. This may be a result of the herpetic infection or possibly of an associated spontaneous encephalitis of rabbits, as it is present in equal proportion at other places in the meninges.

In this case the herpetic infection in the brain as determined by the presence of the intranuclear bodies is limited to the anterior half of the fifth motor nucleus and to a very small focus in the terminal third of the intracerebral portion of the fifth motor nerve root. The left motor nucleus is normal.

Experiment 6.

R. 277. Adult rabbit.

4/26. Right masseter muscle injected with salt solution suspension of material from both eyes of R. 276 (24 hour virus).
4/29. Temp. 104° F.
4/30. Temp. 103.7°.
5/1. Temp. 106°. Etherized. Autopsy negative.

Microscopic Sections. Serial sections through fifth motor root and nucleus show no lesion either in the fifth nerve or in the nucleus. There is no indication of herpetic encephalitis. The temperature reaction of this animal was a little different from the others in that it was above normal (104° F.) on the third day and was extremely high on the fifth (106° F.). No explanation is offered for the failure to find herpetic encephalitis.

Experiment 7.

R. 449. Adult rabbit.

6/9/23. Right masseter muscle inoculated with 24 hour herpes virus from eyes of R. 439 and R. 435.

6/14. Dead. Autopsy negative.

Microscopic sections through fifth motor nucleus. On the right side there is an extensive acute meningitis at the entrance of the motor root into the brain. Brain tissue is destroyed immediately about the bundle of fibers as it enters, and is replaced by inflammatory cells. The meningeal exudate is composed both of polymorphonuclear and mononuclear cells, the latter predominating. There is also considerable fibrin.

The bundle of motor fibers as it passes through to the interior of the medulla is greatly swollen containing large spherical spaces. It appears to be very edematous. At foci along its course are small collections of mononuclear and polymorphonuclear leucocytes in the neighborhood of which are cells, apparently neuroglial, containing intranuclear bodies. To either side within the surrounding brain tissue there is a moderate mononuclear infiltration, and a perivascular infiltration with similar cells, but no intranuclear bodies are found here.

Serial sections were not made but sections through the central portion of the nucleus show a most intense change. Every ganglion cell is necrotic, many completely replaced by polymorphonuclear leucocytes, others contain typical intranuclear bodies, others are faded and vacuolated. There is a general edema and cellular infiltration with polymorphonuclear and mononuclear leucocytes, the latter predominating, but the former phagocyting the dead cells. Numerous neuroglia cells contain intranuclear bodies. Neuroglia cell nuclei containing intranuclear bodies and an accompanying cellular infiltration extend in a zone about the motor nucleus. In bundles of transverse fibers, extending across the midline on a level with the right nucleus, there are small foci of leucocytes with a few neuroglia cells containing intranuclear bodies. These can be followed to the opposite fifth motor nucleus on the left. Here there is also an extensive herpetic infection, but evidently more recent than on the right side. There is scarcely any cellular infiltration, but a great many of the neuroglia cells and most of the ganglion cells show intranuclear bodies of various stages of development, and chromatolysis. There is no neurophagocytosis. The infection as thus indicated is limited to this nucleus and to certain points along the corresponding bundle of motor fibers. The infection on the left side is fairly sharply limited to the motor nucleus though about the periphery there are small foci of infection in other groups of cells. There is no acute meningitis on this side and the cerebellar and pontine tissue elsewhere in the section seems to be free of lesions.

Medulla. Sections through the medulla show the infection has extended throughout on either side near the center of each hemisegment of the medulla. In this area on each side are numerous neuroglia cells containing intranuclear

bodies and some cellular infiltration and perivascular infiltration. The sensory tracts show no change.

The necessity of obtaining for this study the brain of an infected rabbit immediately after the onset of fever is illustrated by the rapid and diffuse spreading of the infection in the pons and medulla in Experiment 7. At such a stage it is not possible to determine with certainty the earliest lesion. It is of interest to observe in this case that an extension of the infection directly across the midline of the pons from the right fifth motor nucleus to the corresponding nucleus on the left. It seems possible that this extension took place along nerve fibers which may associate the two nuclei.

There is no adequate explanation at hand for the negative result in Experiment 6. The fact that the temperature became slightly elevated on the third day makes it seem possible that the febrile reaction resulted from the initial infection in the masseter muscle. If this is true, it is unique in our experience. The possibility of an intercurrent infection was not excluded.

Experiment 4 differs from the first three in that there was no elevation of temperature until the seventh day after injecting the masseter muscle, and in the fact that there is in addition to the extensive lesion in the motor nucleus on the inoculated side a neuritis especially marked just central to the plane at which the sheath of Schwann is lost. This is similar to the lesion which occurs in the intradural portion of the sensory division of the fifth cranial nerve following inoculation of the cornea. It has been shown in previous experiments that following inoculation of herpetic virus into muscles of the hind leg a neuritis of the sciatic nerve may occur in case the animal lives for several days after the inoculation. Apparently the virus may destroy a nerve fiber, penetrate the myelin sheath, and become liberated, producing a local lesion within the nerve under certain circumstances which will be considered later. Such a neuritis is illustrated in Experiment 5. In these experiments, however, the spread of the virus to the motor nucleus was evidently within the myelin sheath as there was a long interval between the motor nuclei and the immediate intradural lesion of Experiment 4 and the extradural lesions of Experiment 5, in which the nerve bundles showed no evidence of herpetic infection either by the presence of intranuclear bodies within neuroglia cells or by a cellular exudate, yet within the confines of the nuclei there was in each an extensive infection with

destruction of ganglion cells, but no greater in extent than in cases showing no neuritis. Experiment 4 may serve as a control for 1 and 2 in that it demonstrates the susceptibility of the tissue about the proximal portion of this nerve if virus escapes from the myelin sheath.

In Experiments 1 and 2 the conditions of the test were satisfactory and the result fulfilled expectations based upon the hypothesis that the virus traverses the nerve along axis-cylinders. In these animals serial sections through the fifth cranial nerve distal and proximal to and including the Gasserian ganglion show no evidence of a herpetic lesion. Minor changes in axis-cylinders could not be detected by the histologic methods used. There were, however, no intranuclear bodies or cellular infiltration throughout the extradural portion of the motor division of the nerves. In the intradural portion proximal to the plane of Schwann's sheath there was likewise no evidence of a lesion. Almost throughout its entire extradural extent the motor nerve is in close proximity to sensory fibers yet there was no escape of virus into the sensory nerve sufficient to produce a lesion in the proximal termination of its fibers where the tissue is very susceptible to the virus.

Throughout the intracerebral course of the nerves in these two experiments there was no evidence of a lesion until the terminal third of the motor bundle was reached just before the fibers disperse to enter the nucleus. Here the myelin sheaths are thin and diminishing in size so that virus contained within them might be permitted to escape, infecting as it did so neighboring neuroglia cells as indicated by the intranuclear bodies of Lipschütz. Had the virus been present in perineural spaces or had it escaped from the myelin sheaths peripheral to this point undoubtedly a local herpetic lesion would have resulted as was demonstrated in Experiment 4. But the few neuroglia cells infected in the terminal portion of the nerve are insignificant compared with the extent of injury and reaction within the nucleus itself. In every case where herpetic encephalitis has followed inoculation into the masseter muscle ganglion cells within the corresponding nucleus have been shown to be necrotic or to contain herpetic intranuclear inclusions, and in these two cases it seems evident that the virus goes directly to the ganglion cell by means of its axis-cylinder. The lesion is not confined to the point of entrance of the fibers into the nucleus, but ganglion cells here and there are

picked out showing in earliest stages intranuclear inclusions and chromatolysis often without an apparent infection of neuroglia cells immediately about. In both cases, however, as was to be expected, neuroglia cells in certain places within the nucleus do contain herpetic inclusions. In each case there was a mild exudation of large phagocytic mononuclear cells with indented nuclei scattered irregularly (Experiment 1) or in addition occurring in groups (Experiment 2). There were no polymorphonuclear leucocytes and no hemorrhage. About small blood vessels within the nucleus were gathered a few mononuclear leucocytes of the type present in the tissue. While many neuroglia cells in each instance contained intranuclear inclusions, none was found in a necrotic state. On the other hand, several necrotic ganglion cells were observed in each case.

The number of ganglion cells infected conformed to what one might expect from an extension of the infection along axis-cylinders. In inoculating the masseter muscle virus was injected into several places, yet with the best of success only a relatively few nerve endings probably were infected, the great majority of those in this muscle remaining out of contact with the virus. Microscopic sections of an inoculated muscle show only scattered foci of inflammation. Under these circumstances the virus could be carried only by relatively few axis-cylinders. If the virus were taken up by perineural fluid and transplanted passively to the brain, it would in all probability regularly infect the cells of Schwann's sheath before it reached the brain, and then of necessity would come in contact with susceptible neuroglia cells, producing its most intense effect as the nerve entered the brain perhaps without reaching the nucleus at all or only later. Were the virus growing in perineural fluid and extending inward by propagating itself, a similar result though more intense would be expected. The passage of the virus directly from the inoculated muscle to the motor nucleus without evidence of a lesion until the nucleus is reached and there picking out ganglion cells almost specifically leaves little room for doubt that the mode of transit is by way of axis-cylinders. It is difficult to conceive of a passive transportation of the virus within axis-cylinders and considerable evidence is at hand that herpetic virus may proliferate within cells. It is consequently believed that the virus grows within the axis-cylinders until it reaches the body of the cell itself, which it destroys, usually penetrating and proliferating within the nucleus

before death of the cell occurs. The liberated virus then spreads to the surrounding neuroglia.

Various stages in the disintegration of ganglion cells may be observed. The earliest is the appearance of irregular masses or a single large mass of acidophilic material within the nucleus associated with an irregular nucleolus. It is evident that the acidophilic material arises within the nucleoplasm and is not derived from the nucleolus. In the earliest stages in which these herpetic inclusions were recognized there was also an obvious chromatolysis. Later the entire nucleus may be filled with a finely granular acidophilic mass. The nucleolus disintegrates and disappears; chromatin particles appear about the nuclear membrane; chromatolysis is completed. The cell thus disintegrates and is phagocyted by polymorphonuclear or mononuclear leucocytes. Cells once entered by the virus as indicated by nuclear inclusions do not recover but invariably undergo necrosis. In early lesions, as illustrated in Experiments 1, 2 and 3, neurophagocytosis of ganglion cells was not observed. In Experiment 7, most of the ganglion cells on the right side were being thus removed, this animal having lived longer, and the lesions were considerably more advanced.

Herpetic Neuritis. It has previously been shown that a neuritis of the sciatic nerve may follow inoculation of herpetic virus into muscles of the hind leg of a rabbit, though we were not able in certain cases to demonstrate a neuritis of the sensory portion of the fifth cranial nerve following inoculation of the cornea.

Marinesco and Draganesco [4] have demonstrated an inflammatory reaction within and about the ciliary nerves and in the ciliary and Gasserian ganglia following herpetic keratitis, and have assumed that herpetic virus enters the central nervous system by propagating itself from a peripheral focus of infection through lymphatic and perivascular spaces of the nerve to the brain.

Experiments presented above demonstrate that the virus may enter the motor nucleus of the fifth cranial nerve following inoculation of a masseter muscle leaving no discovered trace in the motor nerve of its passage. Yet as shown in Experiments 4 and 5, a herpetic neuritis may occur under the conditions of this experiment and a study of the neural lesions in serial sections of the nerve in Experiment 5 has shown clearly how they may occur; and the pathogenesis of the lesions contributes further evidence in our opinion

that the herpetic virus does not propagate itself along perineural and perivascular spaces to enter the brain, but invades the brain through axis-cylinders.

The earliest stage of herpetic neuritis is to be found in a cell of Schwann's sheath, and in the underlying axis-cylinder. Before there is a cellular infiltration about the nerve fiber, the nucleus of a neurolemma cell may be found to present the changes characteristic of a herpetic infection. The chromatin becomes concentrated in a thin broken line peripherally upon the nuclear membrane, while the interior of the nucleus is filled almost completely by a homogeneous or very finely granular eosin staining material. The underlying axis-cylinder appears very faintly granular, vacuolated, and takes a light eosin stain. Sometimes it appears fragmented. Following these changes mononuclear phagocytic cells accumulate about the nerve fiber and may penetrate to the interior of the fiber, and seem to phagocyte particles of the disintegrating axis-cylinder. There is at the same time an accumulation of similar cells about neighboring small blood vessels from which the phagocytes possibly migrate. Occasionally a polymorphonuclear leucocyte is found in the exudate.

The evidences of inflammation of the nerve in the form of a cellular exudate depend upon an infection of cells of the neurolemma by the virus of herpes, and the local injury produced as a result.

The fact that cells of the neurolemma are thus susceptible to infection by the virus of herpes immediately suggests a possible pathway for the virus to propagate itself intracellularly from one neurolemma cell to another from the periphery to the central nervous system without necessarily invading axis-cylinders, for these cells are possibly contiguous from the periphery to the intradural space. This would seem a more acceptable hypothesis than that the virus propagates itself only in perineural spaces. But histological evidence does not support such a conclusion. A study of the serial sections of the motor nerves from Experiments 1 and 2 has revealed no indication of herpetic infection of neurolemma cells, or of inflammation of any kind, yet virus traversed the nerve and through it entered the motor nucleus, destroying ganglion cells.

In the serial sections of the nerve from Experiment 5, inflammatory foci and cells of Schwann's sheath containing intranuclear herpetic bodies are not numerous and occur focally along single nerve

fibers. They remain local, and wide areas of nerve in its longitudinal axis may be free from evidences of injury.

In the event that herpetic virus propagated itself through perineural lymph spaces, it is hardly possible that cells of the neurolemma would escape infection in any instance, and if such infection did occur, there would regularly result an inflammatory focus.

Another explanation for infection of the cells of Schwann's sheath and the resulting neuritis in Experiments 4 and 5 is that virus from an infected axis-cylinder along which it was propagating itself entered these cells from within the myelin sheath to which the neurolemma cells are closely applied. That this occurred is indicated by the fact that axis-cylinders lying beneath the neurolemma cells, which contain herpetic inclusions, present evidences of disintegration, and it is certain that the center of origin of a focus of herpetic neuritis is an individual nerve fiber. It is about such a fiber that inflammatory cells accumulate and following its degeneration phagocyte its remains. Perivascular infiltration and the appearance of inflammatory cells between neural fibers is a secondary reaction to the injury resulting from an herpetic infection within the fiber and of one or more overlying cells of Schwann.

The possibility of an ascending herpetic neuritis resulting from a succession of focal infections of the susceptible cells of the neurolemma, which might reach the brain with a liberation there of the virus, is recognized and can be excluded from a particular case only in the absence of evidences of neuritis as demonstrated in Experiments 1 and 2. It is probable that such a mode of propagation of the virus plays no part in the production of herpetic encephalitis, for herpetic lesions outside nervous tissue do not tend to spread far to infect neighboring cells, but, as frequently demonstrated in the cornea, tend to remain localized in small areas primarily inoculated.

Conclusions

1. Following inoculation of the virus of herpes febrilis into the right masseter muscle, an herpetic encephalitis usually occurs within five to seven days as is indicated by an elevation of body temperature.

2. There is uniformly demonstrable a herpetic lesion in the brain of such animals within the motor nucleus of the right fifth cranial nerve.

3. In early cases no lesion of the corresponding motor nerve may be demonstrated by serial sections until the nucleus is reached.

4. It has been shown that the nerve, especially its intradural portion, is susceptible to infection if the herpetic virus comes in contact with surrounding neurolemma or neuroglia cells.

5. The experiments presented, it is believed, prove that an axis-cylinder transmission of the virus of herpes simplex from a peripheral site of inoculation to the central nervous system occurs and that the virus may be prevented from invading surrounding tissue along the course of the nerve by the myelin sheaths. It seems most probable that the virus grows within the axis-cylinder propagating itself in this way to its central termination, infecting there the highly susceptible cerebral tissue and spreading within the brain in a similar fashion to more distant areas.

6. The possibility of an ascending herpetic neuritis by a propagation of the virus through cells of the neurolemma, which are shown to be susceptible to infection, is recognized, and can only be excluded in the absence of evidences of inflammation and of herpetic inclusions in the course of a particular nerve through which virus has entered the central nervous system.

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DESCRIPTION OF PLATES II–IV

Plate II

Fig. 1. High power of the right motor nucleus of R. 148 showing destruction of ganglion cells, and a cellular exudate.

Plate III

Fig. 2. R. 133. High power of an area in the right motor nucleus showing a necrotic ganglion cell in the center and a normal ganglion cell to the left. The nucleus of the necrotic ganglion cell contains fragmented chromatin and is otherwise filled with eosin-staining material characteristic of herpetic inclusion. There is complete chromatolysis.

Fig. 3. Intranuclear bodies in neuroglia cells within the fifth motor nucleus.

Fig. 4. R. 449. Necrotic motor ganglion cell and phagocytosis of similar cell by polymorphonuclear leucocytes in the right fifth motor nucleus. Note general edema.

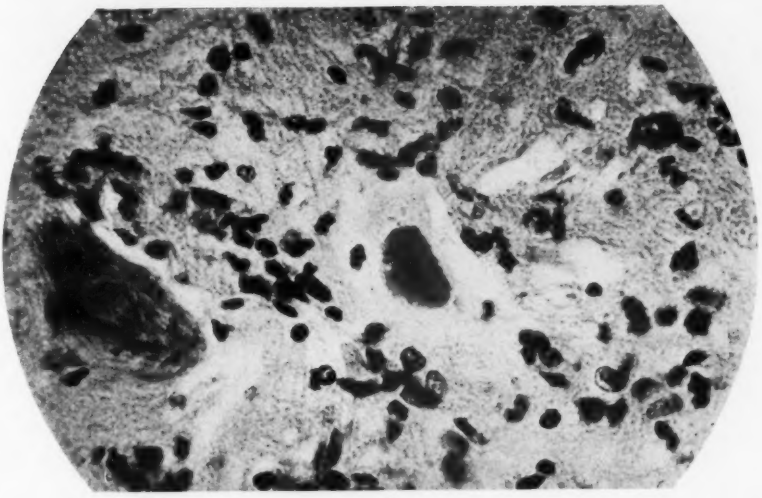
PLATE IV

Fig. 5. R. 224. Drawing to show (*a*) intranuclear body in a cell of Schwann's sheath in the motor division of the fifth cranial nerve. The axis-cylinder (*b*) is faintly granular and vacuolated. The myelin sheath (*c*) appears intact.

Fig. 6. The same nerve showing mononuclear cell exudate about a nerve fiber, and an invasion of the myelin sheath by a phagocytic cell (*a*).

1

Goodpasture Portals of entry to the central nervous system



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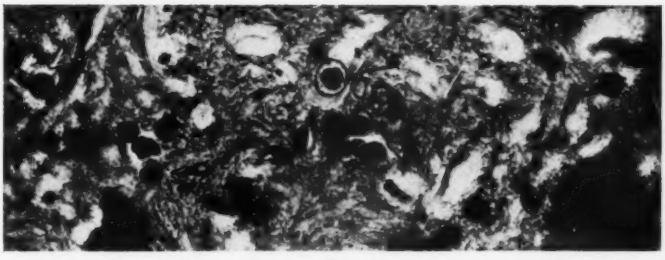

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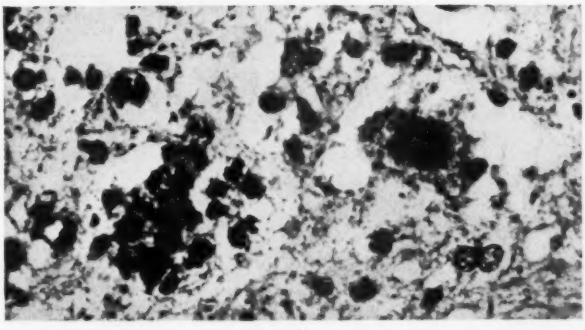

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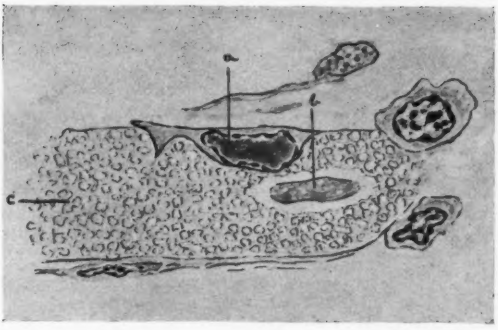
Goodpasture Portals of entry to the central nervous system



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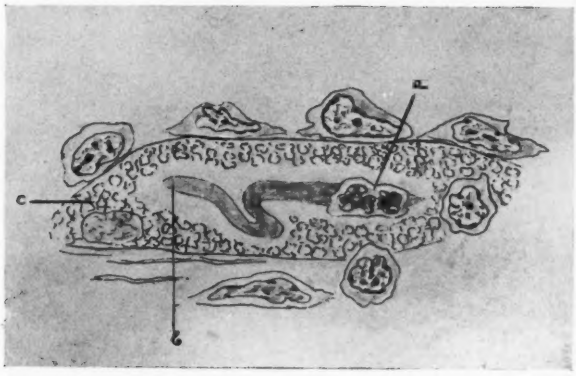

6

THE PATHWAYS OF INFECTION OF THE CENTRAL NERVOUS SYSTEM IN HERPETIC ENCEPHALITIS OF RABBITS CONTRACTED BY CONTACT; WITH A COMPARATIVE COMMENT ON MEDULLARY LESIONS IN A CASE OF HUMAN POLIOMYELITIS*

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PART I

The initial lesion in the central nervous system following a successful peripheral inoculation of rabbits with a strongly neurotropic strain (Strain M) of the virus of herpes simplex is so definitely related to the nerve supplying the inoculated area and its central termination,[1] that given suitable sections of a brain thus infected it is possible for one to determine not only by which nerve the virus gained entrance, but in certain instances in what peripheral distribution of a particular nerve the original infection was located.

By a study of the brain and spinal cord with particular reference to the entrance and central distribution of the cranial and spinal nerves it was thought possible to determine with certainty in this manner the mode of entrance of the virus in cases of contact infection, should rabbits acquire a herpetic encephalitis through exposure to an infected animal.

Normal rabbits proved to be very susceptible to contact infection with Strain M virus and a high per cent of those placed in the same cages with rabbits inoculated upon the cornea contracted in a few days a herpetic encephalitis. The first evidence of an infection of the brain was an elevation of the body temperature. A few animals were permitted to die of the disease and these for the most part showed much the same symptoms without evidence of a unilateral cerebral lesion.

When the body temperature first became elevated there were no other indications of disease. In the course of a day or two, however, there developed muscular weakness and tremors associated with a

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rather general clonic contraction of muscles. One animal turned its head to the right, in others the head was periodically drawn backward as in opisthotonos. These symptoms were rapidly followed by an apparent loss of equilibrium, weakness, convulsions and death. It is probable that a large per cent if not all of our animals that contracted herpetic encephalitis would have died of the disease in a short time though the mortality rate was not determined.

In investigating the paths of entrance of the virus into the central nervous system it was desirable to examine the brain very soon after the virus entered and before lesions other than those associated with the nerve through which the virus was admitted had developed to any great extent.

Experience with herpetic encephalitis following peripheral inoculation and infection had made it appear most certain that in an encephalitis induced through infection by contact the virus would enter the brain in the same manner, that is, along the course of one or more of the peripheral and probably the cranial nerves. With this in mind most of the animals in these experiments were killed from one to four days after the first elevation of body temperature, and the brains fixed immediately after exsanguination by injecting Helly's fluid through the carotid arteries. The brain and spinal cord were then removed in toto and the fixation continued for 24 hours in an ample amount of the same fluid. After washing, blocks including an entire cross section of the brain were cut from the olfactory and frontal lobes, the mid-cerebrum through the infundibulum, midbrain, pons, medulla and spinal cord at various levels. Microscopic sections soon revealed the two localities where herpetic lesions were to be expected, that is, at the entrance of the fifth cranial nerves in the pons and the ninth or glossopharyngeal nerves in the medulla. In succeeding experiments in addition to sections from the above-mentioned regions, serial sections were made including intradural portions of the fifth and ninth nerves and their entire sites of entrance and distribution in the brain.

In this way it has been possible to show that in herpetic encephalitis contracted through contact, bilateral herpetic lesions occur at the central terminations of one or both of these nerves, usually more marked on one side. In the case of the sensory division of the fifth nerve, lesions could be demonstrated within the nerve central to the plane where the sheaths of Schwann disappear and before its

entrance into the brain, as was likewise the case following inoculations of the cornea. However, in the spontaneous or contact infections, the lesion involved a different portion of the nerve, affecting fibers in the dorsal third, whereas following herpetic infection of the cornea the ventral portion of the nerve contained the lesion. This relation was also preserved in the descending crescentic tract, the lesion in contact infections occupying the dorsal horn as the bundle of fibers coursed through the medulla.

In two instances there was a lesion in or at the termination of that group of fibers which enters with the motor division of the fifth cranial nerve and arises from the unipolar ganglion cells situated at the lateral angles of the floor of the fourth ventricle, constituting the mesencephalic or accessory fifth motor nucleus. In one of these cases in which no lesion was present in the corresponding sensory division of the nerve there was no meningitis at its point of entrance. In all the other cases there was an acute meningitis at the entrance of the fifth or ninth nerves or both, as well as an exudate in the brain along their distribution. In those instances where the glossopharyngeal nerves were involved destructive lesions were found in the medulla restricted to the course of these fibers within the medulla and in the corresponding nuclei in the floor of the fourth ventricle.

In earlier stages of the lesions intranuclear herpetic bodies, especially within neuroglia cells, are very numerous, and there is an associated necrosis of cells with many pyknotic nuclear fragments, and a cellular exudate in which polymorphonuclear leucocytes are prominent. In the meninges at this stage there is usually in addition to cellular exudate, fibrin and red blood corpuscles. Fibrinous and hemorrhagic exudate may also be a part of the lesion within the brain.

In each instance it was possible clearly to associate the lesions found with the fifth or ninth cranial nerve. In one brain in which virus evidently entered through the sensory division of the fifth nerve, there was a question as to the origin of an acute herpetic lesion which was found at the site of entrance of one of the glossopharyngeal nerves. It was not possible to determine with certainty whether the latter was due to virus entering through the ninth nerve or to an extension of the infection outward along this nerve from a descending lesion in the sensory tract of the fifth nerve. The cause of confusion was due to the fact that in herpetic encephalitis con-

tracted through contact in which virus enters through the sensory division of the fifth cranial nerve the lesion in the nerve and in its descending tract occupies a dorsal position and passes through the medulla on a level with the ninth nerve as it enters, and only a thin layer of fibers of the Tractus spino-cerebellaris dorsalis [2] separates them from the periphery. However, when virus enters distinctly through the ninth nerve there is not only a peripheral lesion about the fibers of this nerve, but also a well-marked lesion in the corresponding nucleus at the floor of the fourth ventricle.

In later stages of the infection herpetic inclusions in the lesions at the site of entrance were rare or not found at all, the conspicuous feature being the large number of phagocytic mononuclear cells containing vacuoles in their cytoplasm indicating a removal of degenerated myelin sheaths. The initial lesions tend to heal in this way, though the virus extends rapidly to the base of the cerebrum, where its earlier stages of activity may be seen in the cells of the Area praesubicularis of the Lobus piriformis as in R. 221.

In no instance has a lesion in the olfactory lobes been found, and in this series of experiments the olfactory nerves may be entirely excluded from consideration as a portal of entry for the virus.

In most of the following experiments serial sections were made through the entrance of the fifth and ninth cranial nerves.

Experiment 1.

R. 182. Adult male.

4/17. Placed in cage with R. 260 (Herpes both eyes).
4/19. Temp. 103.3° F.
4/20. " 103°.
4/21. " 103°.
4/22. " 103°.
4/23. " 102.4°.
4/24. " 104.2°. On right side of tongue is an irregular ulcerated area about 5 mm. in diameter. Saliva inoculated upon scarified right cornea of R. 273.
4/25. Temp. 103.7° F.
4/26. " 103.4°.
4/27. " ?
4/28. " 100.5°. Very sick and weak. Unsteady equilibrium, difficulty in breathing. Died 1 P.M.
Autopsy negative. Brain shows a macroscopic hemorrhage in medulla on right side. Lesion on tongue healed.

R. 273. Half-grown rabbit.
4/25. Right cornea scarified and inoculated with saliva from R. 182.

4/28. Right eye greatly inflamed and closed with pus. Herpetic kerato-conjunctivitis.
4/30. Head turning to right, partial loss of equilibrium. Herpetic encephalitis.
5/4. Recovering.

Pons.

Serial sections through entrance of fifth cranial nerve. On the right side just median to the plane at which the sheaths of Schwann are lost and involving the dorsal one third of the root there is a large area of necrosis and cellular infiltration in the sensory bundle of the fifth nerve before it enters the brain. The predominant cell in the exudate is the large mononuclear phagocytic cell filled with small vacuoles. About the entering nerve there is a cellular exudate in the meninges consisting chiefly of mononuclears, though containing an appreciable number of polymorphonuclear leucocytes. The portion of the nerve containing sheaths of Schwann shows no change, and the motor root appears normal throughout. Blood vessels within the pons in the neighborhood of the entering nerve present a thin perivascular exudate of mononuclear cells.

On the left side there is a similar lesion occupying the corresponding portion of the sensory root on this side, though the extent of injury is considerably smaller. There is a local meningitis over the area of injury, and a moderate perivascular infiltration in the neighboring pons.

The pyramidal tracts on both sides, but more conspicuously on the right, are clear and the fibers appear vacuolated while neuroglia cells contain intranuclear herpetic bodies.

Medulla. On the right side occupying the dorsal portion of the descending bundle of sensory fibers of the fifth cranial nerve is a large area of necrosis with much hemorrhage and cellular infiltration, by both large mononuclear phagocytes and polymorphonuclear leucocytes. The lesion extends through to the lateral surface on this side and there is a cellular and fibrinous exudate in the meninges. The nucleus of the glossopharyngeal nerve is not involved. There is a small lesion having a similar situation in the fifth sensory tract on the left side and a slight perivascular exudate on this side.

There is a cellular exudate in the meninges over the ventral surface of the medulla and a perivascular infiltration about vessels dipping into the cerebral tissue.

In this case the virus entered the pons through the sensory portion of both fifth cranial nerves and extended downward bilaterally into the medulla in the sensory tracts. In contrast with the lesion of this tract following inoculation of the cornea the lesion is situated in the upper pole of the crescentic tract, and in the dorsal portion of the nerve root before it enters the brain.

The absence of involvement of the region of the glossopharyngeal nucleus excludes any probable entrance of virus through this nerve.

Experiment 2.

R. 221. Adult rabbit.

3/20. Placed in cage with R. 219 (Herpes right eye, inoculated 3/19).
3/21. Temp. 102.5° F.
3/24. Temp. 103°.
3/28. Temp. 105.1°. Nervous and shaky. Rapid respiration. Nose, mouth and eyes negative.

3/29. Temp. 104.5°. Holds head to right.

3/31. Temp. 105.3°. Holds head to right. Bloody discharge from rectum. Has lost considerable weight, salivated. Etherized. Autopsy showed acute intersusception of rectum. There are small hemorrhages superficially along the medulla on the right side.

Pons.

Serial sections through the pons at the entrance of the fifth cranial nerve show no lesion in the nerve on either side. There is slight cellular infiltration about the lateral surfaces of the floor of the fourth ventricle and slight perivascular infiltration. This area of inflammation on either side involves the accessory motor nucleus of the fifth nerve. No herpetic inclusions found.

There is an acute lesion along certain fibers of the right motor bundle characterized by edema and cellular infiltration with large phagocytic mononuclears containing small vacuoles. This occurs first about opposite the main motor nucleus and the cellular infiltration extends upward to a group of cells at the angle of the floor of the fourth ventricle. There is a lesser cellular infiltration similar in distribution on the left. The main motor nucleus with its multipolar ganglion cells is not involved on either side except for moderate perivascular infiltration on the right. No herpetic inclusions are observed, though the acuteness of the lesion and its general appearance indicate its herpetic origin.

Section of the medulla on a level with the entrance of the glossopharyngeal nerve shows at the point of entrance of this nerve on the right side a large acute destructive herpetic lesion with necrosis, hemorrhage, and cellular infiltration. Large mononuclear phagocytes containing vacuoles predominate, though there are many polymorphonuclear leucocytes. This lesion extends inward and dorsally across the fifth sensory tract for a considerable distance along the course of the ninth nerve, the fibers of which are destroyed. There is an interruption of the massive destruction separating the more peripheral lesion from the area of the ninth and tenth nuclei laterally at the floor of the fourth ventricle. This area shows a widespread but circumscribed destructive lesion. The entire area stains lightly and there is destruction within it of nerve fibers and neuroglia, most of the ganglion cells escaping. An abundant cellular exudate is present consisting for the most part of large mononuclear phagocytes. There is also a thick mantle of mononuclear cells about the blood vessels.

On the left side in the same sections there is a lesion of the same distribution though not so extensive and destructive.

Serial sections anterior to this region show a continuation of the area of necrosis and infiltration at the floor of the fourth ventricle but becoming smaller and more deeply situated. It comes to lie rather deeply in the medulla and more laterally, finally disappearing altogether further anteriorly. There is no evidence of a primary lesion in the descending tracts of the fifth nerve.

Posteriorly from the point of entrance of the ninth cranial nerve, the lesion at the floor of the fourth ventricle extends throughout the limits of the tenth nucleus, but diminishing in severity.

The sections indicate that the virus entered the brain in this case principally if not altogether along the glossopharyngeal nerves producing a lesion at their entrance and involving the nuclei of the ninth and tenth nerves on both sides.

In the previous sections the lesions are largely in a state of repair by phagocytosis.

Cerebrum through the infundibulum. Sections through the cerebrum on a

level with the infundibulum show an extensive acute herpetic encephalitis. There is a cellular exudate in the meninges about the infundibulum.

The acute herpetic lesion is sharply circumscribed on each side and more advanced on the right side. The cortical cells of the Area praesubicularis of the Lobus piriformis [2] contain intranuclear herpetic inclusions of various sizes and on the right many of these cells are undergoing necrosis and are calling out a mononuclear cell exudate. The area involved is sharply limited by the Fissura rhinica. On the left side the herpetic lesion has an identical distribution, but intranuclear bodies are less numerous and there is as yet very little necrosis. Sections through the olfactory lobes and tracts show no lesion.

Experiment 3.

R. 245. Normal large adult non-pregnant female.

4/3. Placed in cage with R. 242 (Herpes right eye, inoculated 4/3).
4/4. Temp. 102.8° F.
4/5. " 102.8°.
4/7. " 103.3°.
4/8. " 103°.
4/9. " 103.1°.
4/10. " 104.9°. Eyes clear. No discharge from nose.
4/11. " 103.7°.
4/12. " 102.4°.
4/14. " 105.8°. Eyes, nose and mouth clear. Very nervous, clonic contractions of muscles, sick. Etherized. Pharynx, naso-pharynx, larynx, buccal mucosa, trachea, esophagus and stomach are smooth and pale, showing no evidence of inflammation. Autopsy negative.

Olfactory Lobes. Sections through the olfactory lobes and tracts show no lesion.

Cerebrum. Sections through the cerebrum passing through the infundibulum show no lesion.

Pons. Sections through the pons at the entrance of the fifth nerves show a large destructive herpetic lesion occupying the position of the dorsal third of the sensory division on the right side central to the plane where Schwann's sheath is lost. There is destruction of fibers and myelin sheaths, and a cellular infiltration, with mononuclear phagocytes predominating.

Medulla. Serial sections through the medulla at the entrance of the ninth and tenth cranial nerves show a small herpetic lesion in each descending tract of the fifth cranial nerve. There is also a slight cellular exudate and perivascular infiltration in the nuclei of the ninth and tenth nerves at the floor of the fourth ventricle, but no lesion in relation to these nerves as they enter the medulla.

Cervical Cord shows no lesion.

In this case the virus entered the brain mainly through the sensory division of the right fifth cranial nerve, and probably to a less extent through the left. Possibly virus entered also to a certain extent through the ninth nerve.

Experiment 4.

R. 246. Large adult non-pregnant female.

4/3. Placed in cage with R. 244 (Herpes right eye, inoculated 4/3).
4/4. Temp. 102.8° F.

4/5. Temp. 102.8°.
4/7. " 103.1°.
4/8. " 102.8°.
4/9. " 103.1°.
4/10. " 102.7°.
4/11. " 103°.
4/12. " 103.8°.
4/14. " 105.1°. Eyes, ears and nose clean. No noticeable symptoms. 3 P.M. Temp. 106.4°, respirations rapid. No evidence of muscular involvement. Etherized. Autopsy negative.

Sections through the olfactory and frontal lobes, cerebrum at the infundibulum, and cervical spinal cord show no lesions.

Pons at entrance of fifth nerve. On the left side occupying the dorsal third of the sensory division of the fifth nerve just central to the plane where the sheaths of Schwann are lost, there is a large acute herpetic lesion showing destruction of fibers and myelin sheaths, and cellular infiltration principally with large mononuclear phagocytes filled with vacuoles. There is also a moderate mononuclear cell exudate in the meninges over this area. There is no lesion of the motor root. From the point of entrance into the pons of the fifth nerve upward and inward to the angle of the floor of the fourth ventricle there is a mononuclear perivascular infiltration; and involving the region of the accessory motor nucleus, the Nucleus ventralis Bracchii conjunctivi and the Bracchium conjunctivum cerebelli there is a diffuse cellular infiltration and focal groups of large mononuclear phagocytes.

On the left side there is no lesion in the sensory division of the fifth nerve. In the motor nerve, however, in the bundle of fibers which passes beyond the main motor nucleus to end in the accessory motor nucleus at the angle of the floor of the fourth ventricle, there is an acute herpetic lesion at a point just dorsal to the main motor nucleus. Here there are numerous neuroglia cells containing intranuclear bodies and an infiltration with mononuclear phagocytes. There is also a perivascular infiltration in the neighborhood of the lesion.

Sections through the pons at the entrance of the eighth nerve show only a lesion in the dorsal horn of the crescentic sensory tract of the fifth nerve in the left side, a descending continuation of the lesion in the nerve at its entrance. There is no similar lesion on the right side.

Medulla. Serial sections through the medulla show a moderate cellular infiltration of the median portion on each side and here and there a focus of leucocytes. At the entrance of the glossopharyngeal nerve in the left side there is a destructive herpetic lesion extending inward along the course of entering fibers through the sensory tract of the fifth nerve. About the entrance of the nerve there is a cellular exudate in the meninges.

With a narrow interruption the lesion involves the ninth and tenth nuclei at the base of the fourth ventricle. Here the lesion is largely interstitial consisting of a destruction of fibers, myelin sheaths and neuroglia, and a cellular exudate and perivascular infiltration.

A similar though less extensive lesion involves the entrance of the ninth nerve on the right side, and the corresponding nucleus on this side.

In this case virus entered the pons through the sensory division of the left fifth cranial nerve, and probably through those fibers of the motor divisions of this nerve on each side which terminate in its mesencephalic or accessory nuclei.

In the medulla, virus entered through the glossopharyngeal nerve on each side producing the greater injury on the left side.

Experiment 5.

R. 247. Normal large adult male.

4/3. Placed in cage with R. 243 (Herpes right eye, inoculated 4/3).
4/4. Temp. 102.9° F.
4/5. " 102.8°.
4/7. " 103.2°.
4/8. " 103.3°.
4/9. " 103°.
4/10. " 103°.
4/11. " 102.4°.
4/12. " 102.6°.
4/14. " 102.4°.
4/15. " 102.8°.
4/19. " 102.4°. Remained normal.

Experiment 6.

R. 268. Pregnant white adult female.

4/18. Placed in cage with R. 267 (Herpes right eye, inoculated 4/18).
4/19. Temp. 103° F.
4/20. " 103.1°.
4/21. " 103°.
4/22. " 103°.
4/23. " 103.1°.
4/24. " 103.4°.
4/25. " 103.1°.
4/26. " 102.8°.
4/29. " 103°. Placed in cage with R. 283 (Herpes right eye, inoculated 4/29).
4/30. Temp. 102.8°.
5/1. " 102.8°.
5/2. " 102.8°.
5/3. " 102.5°.
5/14. " 104.8°. Aborted young almost term. Holds head backward, weak and shaky. Emaciated.
5/16. Dead, encephalitis. Autopsy grossly negative.

Experiment 7.

R. 270. Young adult rabbit.

4/20. Placed in cage with R. 269 (Herpes right eye, inoculated 4/20).
4/22. Temp. 102.8° F.
4/23. " 102.8°.
4/24. " 103°.
4/25. " 102.8°.
4/26. " 103.2°.
4/28. " 106°. Etherized. Eyes, ears, nose, and mouth clean. Autopsy negative.

Pons at entrance of fifth cranial nerves. On the right side involving the dorsal half of the sensory division of the fifth nerve there is a large diffuse and very acute herpetic lesion. Innumerable neuroglia cells contain herpetic inclusion-bodies and there is a moderate polymorphonuclear and mononuclear leucocytic exudate and many small hemorrhages. About the entrance of the nerve there is a mononuclear cell exudate in the meninges. No lesion is observed in the fifth nerve on the left, and there is no other lesion in the brain at this level.

Sections of the pons at the entrance of the eighth cranial nerves show an acute herpetic lesion similar to that found in the above sections, occupying the dorsal horn of the right descending tract of the sensory division of the fifth cranial nerve. At this level there is a similar lesion in the corresponding right descending sensory tract. Both these lesions are very acute, presenting numerous cells with herpetic inclusions in their nuclei, and a cellular exudate in which polymorphonuclear leucocytes are prominent.

Medulla. Sections through the medulla including the entrance of the ninth cranial nerve show on both sides an acute herpetic lesion at the entrance of these nerves, and to a lesser degree in the corresponding nuclei at the floor of the fourth ventricle. In the meninges at the entrances there is an acute exudate of mononuclear and polymorphonuclear leucocytes. Within the medullary tissue along the distribution of the fibers of these nerves, but most marked at the periphery, there is an acute herpetic lesion. Innumerable neuroglia cell nuclei contain herpetic inclusions and similar inclusions are found in neuroglia cells in the ninth nucleus on the right side. In both ninth nuclei there is a moderate diffuse cellular exudate. No lesions are observed elsewhere.

In this case herpetic virus entered the brain through the sensory division of the right fifth cranial nerve, and through both glossopharyngeal nerves. The lesions everywhere are very acute.

Experiment 8.

R. 272. Adult rabbit.

4/25. Placed in cage with R. 271 (Herpes right eye, inoculated 4/24).
4/26. Temp. 103° F.
4/29. " 104°.
4/30. " 104.4°.
5/1. " 104.3°. Etherized. Autopsy negative.

Microscopic Notes. Sections through frontal lobes, cerebrum at infundibulum, pons and medulla show no evidence of herpetic infection of the brain.

This animal did not have herpetic encephalitis histologically. The rapid onset of elevated temperature and the fact that the fever remained low for three days suggests that some other cause than an herpetic infection was responsible for it.

Experiment 9.

R. 278. Adult pregnant female.

4/26. Placed in cage with R. 274 (Herpes both eyes, inoculated 4/25).
4/29. Temp. 102.5° F.
4/30. " 102.4°.
5/1. " 103.9°.
5/2. " 102.6°.

5/3. Temp. 102.8°.
5/14. " 102.6°.
Tested for immunity.
6/4. Right eye inoculated with pus from eye of R. 308.
6/9. Temp. 107.2°. Head drawn strongly to the right.
6/10. Lying in cage on right side. Complete loss of equilibrium.
6/13. Dead. Encephalitis.

Discussion. In the above nine experiments in each instance a single normal adult rabbit was placed in the same cage with a rabbit that had been inoculated on the right cornea, within 24 hours previously, with the virus of herpes simplex and had developed as a consequence a severe herpetic kerato-conjunctivitis with a copious discharge of infectious fluid exudate and pus. In an additional case a mother contracted a fatal herpetic encephalitis from contact with her young similarly inoculated on the cornea. Microscopic sections of the brain proved the herpetic nature of her encephalitis but no attempt was made to study the relation of the lesions to nerves.

Thus of ten experiments in which normal rabbits were placed in contact with rabbits infected in the eye, seven contracted a herpetic encephalitis, and three of these died of the disease, four were killed a short time after the first appearance of symptoms. One rabbit which showed a slight elevation of temperature on the fourth day after exposure was killed two days later and the brain revealed no evidence of herpetic infection. Two rabbits after exposure for several days showed no symptoms of herpetic infection. Six weeks later one of these was inoculated upon the eye with herpetic virus and died nine days later with herpetic encephalitis, thus showing no evidence of immunity to the virus.

Each of the seven animals that contracted herpetic encephalitis showed an elevation of body temperature within a few days after exposure, the average being eleven days. This average is high because of one animal whose first elevation of temperature occurred on the twenty-sixth day, in the others the average was eight days. The three animals that died of the disease lived from two to five days after the first recorded elevation of body temperature.

Five rabbits were killed from one to four days after the onset of fever, and serial sections were made through the pons and medulla, in addition to routine cross sections through other portions of the central nervous system. In these cases it was clearly shown that the virus entered the brain twice through the sensory division of the

fifth cranial nerves, once through the ninth cranial nerves, and twice through both these nerves. In two cases in addition to other lesions there was a herpetic inflammation involving the accessory motor nuclei at the lateral angles of the floor of the fourth ventricles. Virus apparently entered by way of the motor nerve through the fibers which have their origin in these nuclei. The exact peripheral distribution of these fibers does not seem to be accurately established, though according to Terterjanz[3] they supply the tensor palati muscle. The possibility of sensory fibers entering the brain with the motor bundle, along which virus might pass, is to be considered, particularly inasmuch as one usually finds a few scattered ganglion cells within the proximal extra-cerebral portion of this nerve.

In each of the encephalitic brains the lesions were bilateral, either involving both the fifth and ninth nerves on each side, or one on one side and one on the other. The short duration of life in the three animals dying from the disease is to be attributed to the extensive involvement of the medulla.

There can be no reasonable doubt in the above cases, in which the central distribution of the cranial nerves was studied, that the herpetic virus entered the brain along either or both the fifth and ninth cranial nerves. Consequently the peripheral source of the virus must have been in the region of the peripheral distribution of these nerves, that is to say, in the case of the sensory division of the fifth nerve most probably in the mucous membrane of the mouth or nose, and in the case of the ninth nerve in the mucous membrane of the posterior portion of the tongue or of the pharynx.

In one case it was almost certain that the infection originated, in part at least, in a lesion on the right lateral surface of the anterior third of the tongue. In R. 182 five days before death a small ulcer was found at this site, and saliva removed at the same time and inoculated upon the cornea of R. 273 induced the usual herpetic keratitis and symptoms of encephalitis from which the rabbit recovered.

In the other animals no lesion of the oral or nasal mucosa was discovered at autopsy, and at the time of death the lesion on the tongue of R. 182 had healed.

Previous experiments of inoculating herpetic virus into various organs and tissues have forced the conclusion that a peripheral take is a necessary preliminary to an invasion of the central nervous system by the virus. It is assumed likewise in herpetic encephalitis

contracted by contact that there is always an initial herpetic lesion in the mucosa of the mouth, nose, or throat from which virus extends along neural fibers innervating this area, into the brain, though this point has not been thoroughly established. It must be borne in mind that an inconspicuous lesion of the mucous membrane may heal readily, so that it may be overlooked or absent at autopsy, yet virus should be demonstrable in the saliva or naso-pharyngeal secretions before encephalitis becomes manifest. This phase of the problem has not yet been investigated beyond the one experiment above mentioned.

As to the manner of transmission of herpetic virus from one animal to another in contact in these experiments, there are the possibilities that it was acquired through contamination of food with the infected secretions, or from licking the infected eye. Small abrasions of the mucous membrane of the mouth, nose or throat would readily lead to a local herpetic infection of the epithelium. This has been demonstrated by lightly scarifying the mucosa inside the cheek and applying to it infected pus from an experimental herpetic keratitis. Sections of the inoculated mucosa removed 24 hours later showed an area of ulceration about which the epithelial cells contained in great numbers the typical intranuclear inclusions of Lipschütz.

In view of these experiments, herpetic encephalitis may be considered a contagious disease for rabbits which are intimately exposed to the virus. There is probably in every case a primary infection of epithelial cells in the mucosa of the mouth, nose or throat. Depending on the situation of such a primary infection the virus propagates itself along the axis-cylinders of sensory nerves supplying the infected tissue until it reaches the central nervous system, usually by way of the fifth and ninth cranial nerves.

Part II

MEDULLARY LESIONS IN A CASE OF HUMAN POLIO-ENCEPHALO-MYELITIS

The possibility of the passage of the virus of poliomyelitis under experimental conditions from the periphery along nerve fibers to the central nervous system has been demonstrated by the investigations of Flexner and Lewis,[4] and of Levaditi and Landsteiner.[5] If one introduces the virus into a peripheral nerve the animal becomes para-

lyzed first in the muscles supplied by this nerve. Following inoculation of the virus into the naso-pharyngeal mucosa the resulting disease manifests itself first by paralysis of muscles of the neck and upper extremities, or inoculated into the mucosa of the intestine the paralysis is first evident in the lower extremities.

These facts point directly to an invasion of the central nervous system by way of nerve fibers under the conditions of the experiment, yet it has not been determined that a similar portal of entry into the central nervous system is involved under the natural conditions of infection in the human being.

The demonstration anatomically of the relation of early lesions in the central nervous system in herpetic encephalitis acquired by contact to the central terminations of the fifth and ninth cranial nerves suggests that a study of suitable cases, especially those succumbing to the disease in its earlier stages, may establish a similar relation of the initial lesions to certain peripheral nerves as portals of entry for the virus of poliomyelitis in man.

An opportunity was afforded recently to study histologically the brain of a sporadic case of polio-encephalo-myelitis in which lesions are present in the medulla that are strikingly analogous to those present in a similar situation in rabbits which have acquired herpetic encephalitis through an invasion of the brain by way of the glossopharyngeal nerve. Sufficient material was not obtained from this case to make a complete study of the central terminations of peripheral nerves, as the diagnosis at the time of autopsy was not established, and the poliomyelitic nature of the disease was demonstrated only by microscopic sections.

The lesions demonstrated, however, are sufficiently suggestive of a relation to the glossopharyngeal and vagus nerves in the medulla to warrant description. Occasion is also afforded to emphasize the desirability of studying the brains of similar cases from the standpoint of determining the relation of the earliest central lesions to the entrance and proximal distribution of peripheral nerves which may serve as portals of entry for the virus of poliomyelitis, especially the fifth, ninth and tenth cranial nerves.

Patient, J. C., a white boy aged 17 years, entered the hospital complaining of having had "sore throat," and at present an inability to swallow. He had had tonsillitis ten years ago and following this his tonsils were removed.

A little over one week ago he had a slight "sore throat" accompanied by

headache, and he thought he had "grippe." On examination at this time his physician found no demonstrable lesion in the throat or pharynx. His appetite was poor and he had been unable to swallow for seven days. Bowels had been regular. There was a slight cough.

On physical examination the ears and nose were negative. The eyes reacted normally. The throat appeared congested and swollen beneath the soft palate on the right. The breath sounds over the chest were very harsh, especially anteriorly over both sides above. There were many dry rales on expiration. Vocal fremitus and tactile fremitus were increased. Heart sounds regular. Abdomen and extremities were negative.

Ten hours later (10 P.M. on the day of admission) the patient was irrational and restless. Frothy mucus was present in the mouth, and he had some cough and expectoration. On attempting to swallow water it ran from the mouth. There was no stertor or cyanosis. There was a generalized bronchitis, much more marked on the left, and there was dullness at the right base posteriorly. Heart was regular; the abdomen retracted; and the tissues much dehydrated. No muscular paralyses were observed in the throat or elsewhere.

The temperature on admission was 98.6° F., rapidly rising to 104° F. a few hours later.

The patient rapidly became worse, at one time was described as kicking and scratching wildly, and died at 8 A.M., 20 hours after entering the hospital.

While in the hospital the temperature arose from 98.6° F. to 106° F., pulse from 112 to 160, and respirations from 22 to 46 per minute.

Cultures from the throat on admission to the hospital and after death were negative for Bacillus diphtheriae.

Necropsy was performed two hours after death. There was found an extensive acute bronchopneumonia, bilateral in distribution, cultures from which yielded Staph. aureus and Pneumococcus Type IV. Heart's blood remained sterile.

In addition to the bronchopneumonia a small hemorrhagic area was found in the gross specimen of the medulla. A brief description of the brain is as follows: Wt. 1400 gms. The blood vessels of the brain are deeply congested and there appears to be a cloudiness of the fluid about the vessels. On section through the medulla there is a reddish-purple area 2 mm. in diameter present in the central part of the right side. This appears to be an area of hemorrhage and it extends for about 0.5 cm. parallel to the long axis of the medulla.

Portions of the medulla including a part of the area in which the hemorrhage occurred were ground and inoculated into the brains of two rabbits. These animals remained normal.

Microscopic sections of the brain show widely spread lesions which are regarded as those of polio-encephalo-myelitis.

Lesions of this nature are recognizable from the basal ganglia to the cervical cord, but are most conspicuous and destructive in the medulla on a level with the entrance of fibers of the ninth and tenth nerves and their nuclei at the floor of the fourth ventricle.

In the basal ganglia only an occasional focus of large mononuclear phagocytic cells is recognizable, and in the neighborhood of such foci there is a moderate lymphocytic infiltration about small blood vessels.

In sections of the cervical portion of the spinal cord there is a quite marked destructive lesion in the ventral horns, characterized by a general mononuclear

phagocytic cell infiltration, perivascular accumulation of lymphocytes, and destruction with phagocytosis of an occasional necrotic motor ganglion cell.

In the medulla, however, corresponding to the position of the hemorrhage observed in the gross specimen, there is a very acute and profoundly destructive inflammatory lesion involving both sides but more marked on the right.

On this side the most conspicuous change is a large area of necrosis and hemorrhage which has become infiltrated by mononuclear phagocytes with vacuolated cytoplasm, no doubt from the removal of disintegrating myelin. The lesion is thus in the reparative stage, and resembles very strikingly lesions occurring in a similar situation in later stages of contact herpetic encephalitis in rabbits. The lesion is situated a little dorsal to the center of this side of the pons above

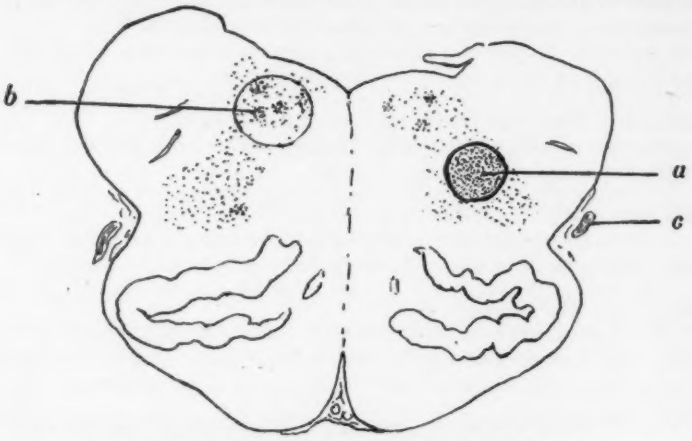

FIG. 1. Schematic drawing of a section through the medulla of a case of polio-encephalo-myelitis. (*a*) is an area of necrosis and hemorrhage. (*b*) is an area of necrosis and cellular infiltration. The dotted areas on each side outline the zones of inflammation corresponding to the distribution of the glossopharyngeal and vagus nerves and their nuclei at the floor of the fourth ventricle. (*c*) is a portion of the glossopharyngeal nerve.

the olive, and includes in its boundaries the approximate position of the Nucleus ambiguus. In addition to this lesion there is an extensive cellular exudate and perivascular infiltration occupying the area of the nuclei of the ninth and tenth nerves on both sides but more extensive on the left side. On this side in addition to a diffuse cellular exudate there is destruction and phagocytosis of ganglion cells within the nucleus.

At this level of the medulla the glossopharyngeal nerves enter in the postolivary sulcus, and although it has been impossible because of insufficient material to trace these nerves completely, there is evident on each side a distribution of cellular exudate and perivascular infiltration from the periphery about the sulci inward and upward to the corresponding ganglia at the floor of the fourth ventricle. On the right side the large area of hemorrhage and necrosis is included in this inflammatory zone. The pathological changes are such as to give a convincing impression that the virus entered the brain through the ninth

or tenth cranial nerves or both, produced its most extensive injury along their central distribution, and spread from this point up and down the cerebrospinal axis. (Text figure 1.)

The extent of the medullary lesions was such as to bring about death of the individual early in the disease. The difficulty in swallowing probably was a result of these lesions and contributed no doubt to the rapid onset of bronchopneumonia which terminated fatally.

In a case of poliomyelitis described by Landsteiner, Levaditi, and Pastia [6] there was evidence that the nasopharynx and tonsils were the portals of entry for the virus. In their case there was an acute inflammation in this region and after death poliomyelitic virus was demonstrated in the tonsillar tissue and in the nasopharyngeal mucous membrane by inoculations into monkeys, but none was demonstrable in cervical lymph nodes or salivary glands.

While in the majority of cases of poliomyelitis lesions in the medulla are undoubtedly not so evident or extensive as in the one we have described, it is nevertheless possible that the regular route of invasion of the central nervous system is by way of the nerves supplying the mucous membranes of the mouth, nose and pharynx, as in contact infection with herpes. A small local lesion at the site of entry of these nerves into the brain could undoubtedly serve as a center from which the virus would spread rapidly, attacking the nervous structures which are most susceptible to its effects. An analogy is to be found in herpetic encephalitis where an initial lesion at the site of entry of a particular nerve may be small and in a state of repair, while a diffuse acute infection of the basal cerebral cortex leads to death of the animal.

Summary

1. Normal rabbits readily contract herpetic encephalitis when placed in the same cages with rabbits inoculated on the cornea with the virus of herpes simplex (strain M).

2. There is probably in every case an initial herpetic infection in the mucous membrane of the mouth, nose or throat.

3. The virus reaches the central nervous system through the sensory division of the fifth and the ninth cranial nerves.

4. Herpetic lesions have been demonstrated microscopically in association with the central termination of these nerves in the pons and medulla.

5. In no instance in these experiments has a lesion been observed in the olfactory lobes, or in association with any other cranial or spinal nerve.

6. A case of polio-encephalo-myelitis in a boy is described in which medullary lesions were found which appear to be directly related to the central distribution of the ninth and tenth cranial nerves. It is suggested that the virus of poliomyelitis in human infections may enter the brain through peripheral nerves.

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CERTAIN FACTORS DETERMINING THE INCIDENCE AND SEVERITY OF HERPETIC ENCEPHALITIS IN RABBITS *

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In the course of passing the virus of herpes simplex from rabbit to rabbit during the past eighteen months several observations of interest have been made relating to the natural resistance of these animals to herpetic encephalitis. In this paper it is proposed to record such as have been controlled by appropriate experimental tests, especially the facts thus established that have a bearing on the relation of the strain of virus, the size of the peripheral herpetic infection experimentally induced, and the age of the animal, to the incidence and severity of herpetic encephalitis.

It was early recognized that not all rabbits infected with the virus of herpes, even with a highly neurotropic strain, acquire as a result a herpetic encephalitis, and that an encephalitis once acquired is not invariably fatal. It became evident that the incidence and severity of herpetic encephalitis were controlled by two groups of factors, that is (1) those depending upon the virus used, and (2) those depending upon the animal sustaining the infection. It has been possible to analyze certain of these factors in each group, which for convenience may be described under the following headings.

I. FACTORS DEPENDING UPON THE VIRUS

(1) *Virulence of the strain of virus.* With this particular virus virulence so far as its propensity for invading the central nervous system and inducing encephalitis is concerned may be regarded as synonymous with what has been termed its "neurotropism," a characteristic which is poorly understood, but which in final analysis may be found not to be a specific affinity of the virus for nervous tissue, but a property dependent upon conditions which render cells of the central nervous system more readily invaded by certain strains than by others. It has been shown for instance that a strain of the

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virus of herpes simplex which was never observed to cause an encephalitis following a peripheral infection, was nevertheless able to proliferate locally following primary inoculation not only in nervous tissue but in derivatives of all three embryonic cellular layers, ectoderm, endoderm, and mesoderm;[1] and as will be shown below, an infection upon the cornea with a highly "neurotropic" strain does not necessarily lead to an encephalitis, provided the site of inoculation is a sufficiently small one. The virus of herpes simplex thus appears to be able to infect the cells of any tissue of the body if a favorable initial injury is induced at the time of inoculation.

Yet different strains of this virus exhibit a notable variation in their capacity to induce herpetic encephalitis from a peripheral infection. This has been demonstrated particularly well by inoculations upon the scarified cornea, using two strains of virus. The one, virus F, regularly produced a purulent kerato-conjunctivitis apparently of an equal intensity to that resulting from an infection with the other, virus M, and yet never leading to an encephalitis. Virus M, on the contrary, has consistently caused an infection of the central nervous system when a uniform technique was followed in inoculating the cornea. Virus F, however, when introduced directly into the brain usually caused a fatal infection, yet not invariably so.

The incidence and severity of an encephalitis are thus determined by some quality of the virus enabling it to penetrate nerves and propagate itself along them to the brain or spinal cord, a quality which varies widely in different strains.

(2) *Size of the initial peripheral infection.* When a uniform technique is followed for inoculating a rabbit's cornea for passage of the virus, consisting in anesthetizing the animal with ether, evulsing the bulbus and making from five to six fairly deep parallel incisions entirely across the cornea and following these with similar incisions at right angles, then inoculating with pus from an eye infected in the same way 48 hours previously, by rubbing the inoculum into the incisions, an encephalitis invariably follows the kerato-conjunctivitis thus induced in adult rabbits, and is fatal in seven to nine days except in certain instances to be mentioned below.

Having established such a procedure, the results of which could be very accurately predicted, it was possible to vary both the conditions of the virus inoculated, using the same neurotropic strain, as well as the size of the area scarified, and in this way to estimate

the relative importance of each. It had been observed, especially when rabbits not full grown were used for transfer, that the inoculation with pus from an eye infected more than 48 hours previously resulted sometimes in an encephalitis which was not fatal, and for each 24 hours succeeding the first 48 hours the chances of inducing a fatal encephalitis became rapidly less, so that usually it was difficult to induce even an herpetic keratitis, using for the inoculum the pus from an eye infected five days previously.

This rapid reduction in the virulence of pus from an infected eye is due, we believe, to a diminution in the quantity of active virus and is directly proportional to the presence of cells, in the inflamed eye, which contain herpetic nuclear inclusions. These inclusions have been regarded by Lipschütz and by Goodpasture and Teague as representing the presence and growth of the virus within the nucleus.

At the end of the first 24 hours after inoculation the virulence of the secretion from the eye is apparently at its maximum, a time coincident with the full development of the clear herpetic vesicles along the lines of scarification. There is little or no exudate from the eye at this time, and it is, therefore, more convenient for practical purposes to use virus contained in pus 24 hours later or 48 hours after inoculation, as this is easily manipulated, and may be preserved in a convenient form in dilute glycerol.

It has been observed that an inoculation of the cornea with pus removed from an eye 72 hours or later after primary infection usually induces a milder keratitis as well as a less severe attack of herpetic encephalitis, and this is assumed to be due to a diminution in the amount of virus present in the exudate with a resulting infection only at certain points along the lines of scarification and a tendency for the infection to remain localized in these particular areas of corneal epithelium. The following experiments were performed to test this point.

Experiment 1. Two adult non-pregnant rabbits were inoculated on the right cornea in a scarified area 1 mm. square, near the corneal margin in the upper quadrant, with 24 hour virus from the eye of R. 297. A third rabbit was inoculated with the same virus after scarifying the cornea in the usual way for passage, as a control. The control died on the ninth day after the inoculation, with herpetic encephalitis. One of the two other rabbits died of encephalitis on the fourteenth day, the remaining one recovered after a severe attack of encephalitis.

This experiment shows that the virus from an eye inoculated 24 hours previously may produce a fatal encephalitis following even a very small initial inoculation on the cornea, but not constantly.

Experiment 2. Two adult male rabbits were inoculated on the right cornea in the center by making a fairly deep incision 2 mm. in length and patting into the incision a drop of pus removed from an eye inoculated 48 hours previously. As a control a third rabbit was inoculated with the same pus on the right cornea after scarifying in the routine way for passage.

The control rabbit died of herpetic encephalitis nine days later.

Neither of the other two rabbits showed an elevation of body temperature, turning of the head or any evidence of encephalitis. Typical vesicles, however, developed at the site of inoculation within 24 hours and two days later a secondary crop of vesicles appeared in the upper quadrant of the eye of one rabbit and in the posterior quadrant of the eye in the other rabbit. There was a mild conjunctivitis with congestion and lacrimation, but little pus. Ten days after inoculation the eyes were completely healed and 30 days later these animals were found to be immune to corneal infection with the same virus.

This experiment shows that notwithstanding a local herpetic lesion on the cornea, virus M does not necessarily invade the central nervous system, provided the initial site of scarification is small enough and 48 hour virus is used. The herpetic infection exhibits little tendency to spread locally, remaining quite sharply confined to the area inoculated. Isolated vesicles, however, do appear within areas corresponding apparently to the drainage by the corneal lymph spaces.

A recognition of this limitation of the local infection is of importance in understanding the nature of a local herpetic lesion, and of the manner in which the virus extends to the central nervous system. The infection tends to remain localized immediately to the cells about the point injured. The virus proliferates just as actively and as abundantly at the site of a small incision as it does proportionally in more extensive scarifications, but evidently the initial injury and infection must involve a certain minimum area or number of nerve fibers to result in a proliferation of the virus within axis-cylinders and along them to the brain to result in an encephalitis. And even though virus reaches the brain a minimum amount or a minimum extent of the initial intracerebral lesion is necessary to cause a fatal infection, for, as it is the transmission of the virus along the fifth cranial nerve from the cornea to the pons which initiates an herpetic lesion in the central distribution of this nerve, so in a fatal infection following corneal inoculation there is a secondary distribution of the virus from this initial lesion in the pons to the cerebrum

which brings about a fatal termination. Presumably if the lesion in the pons is small enough, a fatal encephalitis will not follow.

It is to be noted that in the two sets of experiments above recorded different areas of the cornea were scarified, in the first set an area near the conjunctival margin, in the second an area of approximately the same size in the center of the cornea. About the periphery of the cornea there is a rich plexus of nerves, the plexus annularis, from which fibers pass inwardly losing their medullary sheaths a short distance from the corneal margin. An inoculation near the periphery therefore would probably expose to the virus a greater number of nerve filaments than a similar inoculation nearer the center. This anatomical arrangement of the nerve fibers may explain the fact that encephalitis resulted in both animals of the first set of experiments and in neither of the second. It may thus be that of much greater importance than the size of the peripheral inoculation from the standpoint of the incidence of encephalitis is the number of nerve fibers involved and their degree of injury.

On this principle it is possible to immunize rabbits against a highly neurotropic strain of virus by vaccinating them in a small area relatively free of nerves with the same virulent strain, and without a likelihood of inducing thereby an encephalitis.

It may be stated as a result of these experiments that with a particular strain of herpetic virus the incidence and severity of encephalitis following corneal inoculation is within limits directly proportional to the size and extent of the initial corneal infection. This probably means that whether or not an encephalitis results depends on the extent of susceptible nervous tissue actually exposed to the peripheral infection, and on the fact that the virus in a local peripheral focus of infection will not spread far to infect neighboring cells and nervous tissue from which it might proceed to the brain.

II. Factors Depending upon the Individual Rabbit Infected

In the great majority of cases in our experiments herpetic virus has been passed for preservation in an active state from one adult rabbit to another by means of corneal inoculations. Under these circumstances the occurrence of a fatal encephalitis has quite uniformly followed where virus in 24 or 48 hour fresh exudate from an

eye was used for the inoculum after extensive scarification of the cornea. A particular strain of rabbits to be described below presented an exception. Occasionally, however, young animals of various ages have served for transfer, and with these the severity of the encephalitis which always occurred varied very greatly, and several groups of experiments have definitely proved that young rabbits of susceptible parentage are very resistant to herpetic encephalitis and rarely die of the disease, though the extent and severity of the corneal infection is proportionately as great as in adults.

(1) *Age of the rabbit.* To test the severity of the encephalitis following corneal inoculation three groups of young animals in individual litters were used.

Group 1. Three young rabbits of the same litter weighing 600, 600, and 670 grams respectively and about ten weeks of age were inoculated on the scarified right cornea with pus from the eye of a rabbit inoculated 48 hours previously. One of these rabbits died on the seventh day, another on the eighth, another on the ninth day afterward with herpetic encephalitis. The protocol of one will serve to illustrate the group.

R. 106. Wt. 670 gms.
11/24/23. Temp. 103.8° F. Inoculated on right cornea with pus from eye of R. 99.
11/25. Good take. Clear vesicles along lines of incision. Some pus.
11/26. Temp. 104°. Purulent kerato-conjunctivitis.
11/27. " 103.6°. Eye closed with purulent exudate. No turning of head.
11/28. " 105.9°. Head beginning to turn to right.
11/29. " 106.4°. Strong turning to right.
11/30. " 104.5°. Same.
12/1. " 105.4°. Beginning loss of equilibrium, falling to right.
12/2. Lying on right side moribund, grinding teeth.
12/3. Found dead.

With the same virus and at the same time another group was inoculated similarly.

Group 2. Four young rabbits weighing 240 grams each and about four weeks of age were inoculated on the right cornea after extensive scarification, with pus from the eye of R. 99 inoculated 48 hours previously. One of these died of herpetic encephalitis nine days later, the other three acquired an encephalitis but recovered completely. The protocol of one recovered animal is appended.

R. 109. Wt. 240 gms.

11/24/23. Right cornea inoculated.
11/25. Good take. Clear vesicles along incisions.
11/26. Temp. 103.8° F. Purulent kerato-conjunctivitis.
11/27. " 105.8°. Eye closed. Head turning to right.
11/28. " 105.4°. Strong turning to right.
11/29. " 105.2°. Same.
11/30. " 105°. Same.
12/1. " 105.5°. Same.
12/2. " 104°. Seems better. Less turning of head.
12/3. " 102.5°. No turning. Better.
12/4. " 104. 5°. No turning.
12/6. " 103.6°. Salivated. No turning.
12/14. " 103.8°. Completely recovered. Right cornea completely opaque.

An adult rabbit inoculated on the right cornea at the same time as a control for these two groups died on the seventh day after inoculation with typical encephalitis.

Group 3. Seven young rabbits twenty-six days old and averaging 250 grams in weight belonging to the same litter were inoculated on the right cornea with pus from a herpetic keratitis inoculated 48 hours previously. Each of these rabbits contracted a mild encephalitis, and all of them recovered. The following is a typical protocol.

R. 201. Young rabbit 26 days old. Wt. 250 gms.

3/7/24. Right cornea inoculated with pus from right eye of R. 192.
3/8. Temp. 102.7° F. Good take. Clear vesicles.
3/10. " 104.3°. Purulent kerato-conjunctivitis.
3/11. " 106°. Head turning to right.
3/12. " 105.8°. Little turn to right.
3/13. " 103°. No turn observed.
3/14. " 106.3°. Strong turn to right.
3/15. " 105. 7°. Same.
3/17. " 103.5°. Better. No turn.
3/18. " 103.4°. Salivation. No turn.
3/20. " 103.5°.
4/1. Complete recovery. Right cornea opaque.

A control adult rabbit inoculated on the right cornea with the same virus at the same time died nine days later with typical herpetic encephalitis.

These experiments demonstrate that young rabbits up to four weeks of age may acquire an encephalitis from an experimental corneal infection with herpetic virus, but usually in a mild form from which they rarely die. Our experience in this respect is contrary to

that of Ford and Amoss [2] with herpetic infections. They state that young rabbits are more susceptible to infection with herpetic virus than are adults. Adult rabbits, however, have in our hands proved so uniformly susceptible to herpetic encephalitis that in testing the virulence of a certain strain or in attempting to demonstrate the presence of herpetic virus in human or other material, we would not hesitate to use full-grown rabbits in preference to younger ones.

One small group of rabbits of a particular strain have proved to be very resistant to herpetic encephalitis, although they readily contract an infection of the brain following corneal inoculation. These rabbits were bought in a single lot and probably were of the same family.

(2) *Resistant strain of rabbits.* In this lot there were four rather large adult uniformly light brown rabbits of apparently the same size and age. One of them was inoculated upon the right cornea for passage in the usual way with virus in the pus from a 48 hour infection of the eye. An intense purulent kerato-conjunctivitis resulted and was as usual followed by a turning of the head to the inoculated side and a complete loss of equilibrium. The animal remained very sick for a few days, finally recovering. The remaining three rabbits of the lot, and also controls, were similarly inoculated. The controls died in the usual time, but each of the brown rabbits recovered. The following experiment illustrates the group.

R. 244. Non-pregnant large brown female.

4/3/24. Right eye inoculated with pus from eye of R. 240 (48 hour virus).
4/4. Temp. 103° F. Good take on cornea.
4/5. " 103.5°.
4/7. " 105.3°. Head turning to right.
4/8. " 105.8°. Same.
4/9. " 105.1°. Beginning loss of equilibrium.
4/10. " 107.3°. Complete loss of equilibrium. Lying on right side struggling.
4/11. Sitting up in cage, very weak, but recovering.
4/12. Temp. 105°. Same.
4/17. " 105.5°. Unsteady, head drawn to right. Gnashes teeth. Nystagmus left eye.
5/1. Has steadily improved. Impairment of equilibrium.
6/14. Has recovered, but is still unsteady with tendency to turn head to right. Temp. 102.5°.

Two adult rabbits similarly inoculated at the same time served as controls. Both died eight days later with herpetic encephalitis.

While this is a small group of animals from which to draw the inference that a certain breed of rabbits is more resistant to herpetic encephalitis than others, the uniformity of our results with many rabbits of various breeds and mixtures has been, in our opinion, of sufficient constancy to justify this conclusion.

Summary

1. The incidence and severity of herpetic encephalitis resulting from a peripheral focus of infection in rabbits depends upon the virulence, i.e., the "neurotropic" property of the strain of virus, and the size of the peripheral area infected.

2. Rabbits highly susceptible to herpetic encephalitis may be immunized against this infection by inoculating a sufficiently small area of the cornea with a highly neurotropic strain without causing encephalitis.

3. The severity of an encephalitis depends among other things upon the quantity of virus entering the brain through a peripheral nerve.

4. Very young rabbits are strongly resistant to herpetic encephalitis and rarely die following an infection of the brain through a herpetic keratitis.

5. A certain breed of rabbits has been observed which has a stronger natural resistance to herpetic encephalitis than others.

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RIEDEL'S STRUMA ASSOCIATED WITH REMNANTS OF THE POST-BRANCHIAL BODY*

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Enlargement of the thyroid gland due to chronic inflammation is uncommon and when it does occur the preoperative diagnosis is rarely correct. Goitre of this type was first described by Riedel in 1896 and is still designated as Riedel's iron-hard struma.

The subject of the present paper was a man, fifty years old, admitted to the surgical service of Dr. T. H. Russell. The patient complained only of a swelling in the neck, failing eyesight and hoarseness. Family history and earlier personal history were unimportant. The enlargement of the neck had been observed four or five months before and it had gradually increased, without pain. It obviously involved both lobes of the thyroid gland and it was extremely hard. The tonsils were enlarged and there was a severe inflammation of the pharynx and trachea. The preoperative diagnosis was carcinoma.

At operation the tumor mass was removed entire without great difficulty. Postoperative recovery was uneventful.

Two months later the patient's tonsils were removed by Dr. M. F. Jones. One tonsil measured 31 × 20 × 14 mm., the other 21 × 15 × 12 mm. The tonsils showed an increase of lymphoid tissue, irregular fibrosis, thickened septa and capsule. Leptothrix colonies were present in the crypts. In the dense fibrous tissue attached to the capsule there was a large blood vessel filled with an organized thrombus.

Recent reports indicate that the patient has fully recovered his health.

The surgical specimen from the first operation (Fig. 1) presented an almost symmetrical enlargement of both lobes of the thyroid gland and measured 19 × 6 × 4 cm. The external surface presented rounded prominences but was smooth except for delicate fibrous tabs. The capsule was thickened and in it there were numerous con-

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gested vessels. The specimen was very hard and, on section, the cut surface everywhere presented the same appearance, yellowish-white with thin translucent lines between small irregular opaque areas, somewhat suggesting lobules. There was no visible colloid. A mass of lymph nodes, 18 × 7 × 5 mm., was attached to the middle of the anterior, inferior border. These nodes were firm and on section light pink with small hemorrhagic spots.

The histologic structure in no way suggested carcinoma but rather was that of a chronic granuloma. Dr. James Ewing, to whom microscopic specimens were submitted, recognized the condition as a fairly late stage of Riedel's struma. His counsel and stimulating encouragement are gratefully acknowledged.

Sections from all parts of the specimen show a striking change in the thyroid structure (Fig. 2). The capsule is thickened and consists of thin irregular lamellae of fibrous tissue. In some places it contains collections of round cells and plasma cells, in the center of which may be seen a small blood vessel, often with swollen endothelium. A few of these contain polymorphonuclear leucocytes. Wide septa of fibrous tissue extend inward from the capsule into the substance of the gland and fascicles of fibrous tissue cross and recross the intervals between the larger bands in every direction. The fibrous tissue is in part hyaline and structureless and in part composed of fibers between which can be seen the elongated nuclei of the connective-tissue cells. At other places one finds younger fibroblastic cells with long protoplasmic processes. Scattered through this fibrous tissue there are both diffuse and circumscribed collections of small lymphocytes and plasma cells, scattered well-defined lymph follicles and, in widely separated areas, remnants of gland vesicles.

The thyroid parenchyma persists especially just beneath the capsule but is also to be found at various depths (Fig. 3). In these areas one finds thyroid vesicles compactly grouped to form lobules 3 to 10 mm. in diameter. Many of these contain colloid. Lymph nodules encroach upon the lobules and an exudate of lymphocytes and plasma cells is found within the lobule, about the vesicles, between the epithelial cells and also in the lumina. In many places the epithelial lining of the vesicle has disappeared entirely or in part, being replaced by fibrous stroma containing inflammatory cells. This intralobular fibrous tissue is at times so abundant and compact as to be with difficulty distinguished from the interlobular bands.

In other locations the parenchyma persists as isolated groups of epithelial cells embedded in the fibrous tissue without any arrangement suggesting thyroid lobules (Figs. 4, 5 and 6). These isolated portions of epithelium sometimes surround colloid or they may appear as irregular clumps or cellular strands. The individual epithelial cells are well preserved and in them mitotic division figures are occasionally found (Fig. 6).

The well defined lymph nodules are most abundant near the capsule but they are found throughout the substance of the specimen, at least one and often several in each square centimeter of tissue surface. These follicles are especially conspicuous at the margins of the recognizable thyroid lobules (Fig. 3). Such a follicle is from 100 to 900 μ in diameter with lymphocytes at the center and abundant plasma cells in the peripheral zone. Active germinal centers are lacking.

The blood supply is plentiful but is not a striking feature in the pathological picture. Occasionally a vessel with thickened wall is seen.

Rarely in some of the sections peculiar groups of epithelial cells are encountered (Figs. 7 and 8). These cell nests are prominent features and appear to have persisted with only slight change in the midst of the inflammatory transformation occurring in the surrounding thyroid tissue. Such a nest may be found in a lymph nodule (Fig. 7), in an interlobular septum or within the thyroid lobule. As a rule the nest is solitary (Fig. 7) but they may be paired or several may be found in one microscopic field. Several closely related groups of these nests are found just beneath the anterior capsule near the central zone of each lateral lobe (Fig. 8). The nests vary in shape from round and oval masses to irregularly branched groups with thick blunt prolongations. They measure 30 to 200 μ in diameter. The epithelial cells are several rows in depth and the outer row rests upon a definite basement membrane. There is, however, no evident fibrous capsule. These epithelial cells are large and polyhedral with oval vesicular nuclei. In some places the cells are separated by narrow gaps traversed by fine intercellular bridges, suggesting the lymph-canalicular system of squamous epithelium (Fig. 8). When compressed the cells appear flattened and curved about each other (Fig. 7).

A few of these cell nests show lumina, lined by cuboidal or cylin-

drical cells. One larger nest with a lumen appears to possess a duct leading away from it (Fig. 9).

These peculiar cell nests are interpreted as remnants of the post-branchial body, described by Getzowa. Here are found the solid cell nests representing the glandular parenchyma of the post-branchial body (Figs. 7 and 8) and the hollow cell nests with ducts representing the accessory cysts (Fig. 9). The main duct representing the original pharyngeal pouch has not been found.

The post-branchial, or more exactly the ultimo-branchial body, is rarely found in human material. Hermann and Verdun in a study of many human embryos recognized this structure within the thyroid gland in only three, 55, 63 and 95 mm. in length, respectively. They never found it in the adult thyroid.

Getzowa demonstrated the ultimo-branchial body in seven individuals, one an embryo 90 mm. long, another a new-born infant, third, an infant three weeks old, and in four adults (three cretins and one idiot). Pathological overgrowth of these remnants has been described by Getzowa and by Langhans. Berard more recently has reported a branchioma possibly originating in these rudiments.

The possible relation between these post-branchial remnants and Riedel's struma appears not to have been considered.

Riedel waited twelve years before reporting his first iron-hard struma, on account of its unique and inexplicable features. At the end of this period he observed a second similar goitre. Almost immediately Cordua, Tailhefer, Berry and Ricard reported similar cases. These early reports deal chiefly with clinical findings with only scant attention to the structural changes. As a rule the patients had been well until there appeared a painless swelling of the thyroid gland which progressed until the symptoms due to pressure and adhesions compelled them to seek relief.

Silatchek, in 1910, under the title "Peristrumitis Indurativa," added another case and also described the pathological picture in some detail. A report by Spannaus appeared in the same year. Delore and Alamartine, in 1911, reported a new case and collected thirteen in the literature. There quickly followed, in 1912, a report by Murray and a report of four cases observed by Hashimoto. Hashimoto regarded his disease as distinct from Riedel's struma and named it "Struma Lymphomatosa." Later observers, notably Ewing, consider the microscopic findings of Hashimoto to represent

TABLE 1. *Reported Cases of Riedel's Struma without concomitant Tuberculosis or Syphilis*

No.	Author	Year	Sex and Age	Preoperative diagnosis	Duration	Lobe	Remarks	Operation	Sequel
1	Riedel	1894	M 40	Sarcoma	?	Both	—	Partial resection	Recovery
2	Riedel	1894	F 4	Strumitis	?	Both	—	Partial resection	Recovery
3	Riedel	1896	M 42	Carcinoma	6 mos.	Both	Goitre 1 yr.	Inoperable	Death in 15 mos.
4	Riedel	1896	F 23	Strumitis	2 mos.	Both	Mother had goitre	Inoperable	Death in 2 mos.
5	Riedel	1897	M 29	Fibrosarcoma	2 mos.	Right	KI ineffective	Partial resections (2)	Recovery 10 yrs. later
6	Cordua	1896	F 13	Sarcoma	3 yrs.	Right	—	Inoperable	Recovery
7	Tailhefer	1897	M 20	Carcinoma	3 mos.	Left	—	Small resection	R. hemiplegia
8	Berry	1898	F 40	Carcinoma?	5 wks.	Right	Nodular	R. lobe removed	Recovery
9	Ricard	1901	M — †	Carcinoma	?	Both	—	Total extirpation; 4th nerve cut	Fistula of thoracic duct. Recovery
10	Silatchek	1910	M 32	Peristrumitis indurativa	4 yrs.	Right	Father had goitre; KI ineffective	Exploratory incision X-Ray	Tardy recovery
11	Spannaus	1910	M 52	Carcinoma	18 mos.	Both	Goitre 7 yrs; KI ineffective	Partial resection	Recovery
12	Delore and Alamartine	1911	M 29	Carcinoma? Thyroidite ligneuse	2 mos.	Right	Goitre 1 yr.	Resection; jugular vein cut	Death in 4 days
13	Murray	1912	M 23	Carcinoma?	3 mos.	Both	Goitre 18 mos.	Partial resection	Recovery; myxedema *
14	Hashimoto	1912	F 61	Parenchymatous goitre	7 mos.	Both	Good health	Partial resection	Recovery; myxedema *
15	Hashimoto	1912	F 40	Carcinoma?	6 wks.	Both	Good health	Partial resection	Recovery; myxedema *
16	Hashimoto	1912	F 55	Struma fibrosa	4 wks.	Both	Good health	Partial resection	Recovery; myxedema *
17	Hashimoto	1912	F 45	Struma fibrosa	?	Both	Good health	Partial resection	Recurrent goitre
18	Meyer	1913	F 25	Malignancy	9 mos.	Right	Goitre; KI ineffective	Partial resections (2)	Pneumothorax; death
19	Heinike	1914	F 50	Carcinoma	1 yr.	Both	KI and arsphenamine	Complete extirpation	Myxedema *
20	Heinike	1914	F 47	Carcinoma	1 yr.	Both	Goitre 2 yrs.	Partial resection	Recovery; X-Ray
21	Reist	1922	F 40	Carcinoma?	Brief	Both	Good health	Removal	Recovery; myxedema *
22	Reist	1922	F 41	Carcinoma?	?	?	Good health	Removal	Recovery
23	Reist	1922	F 50	Sarcoma?	1 yr.	Right	Goitre 5 yrs.	Removal	Recovery
24	Reist	1922	F 60	Carcinoma?	4 mos.	Left	—	Removal	Recovery after 1 yr.
25	Meeker	1923	M 50	Carcinoma	5 mos.	Both	Good health	Removal	Myxedema slight

* Myxedema following thyroidectomy was successfully treated with thyroid.

† A young person, age not recorded.

an early stage of Riedel's iron-hard struma. In 1913, Meyer reported a malignant granuloma of the thyroid gland, which was later classified as Riedel's struma. In 1914, Heinike reviewed the literature under the title, "Chronic Thyroiditis." In the same year, Monod in a Paris thesis reported three cases of Riedel's struma which he believed to be due to syphilis.

The only references to Riedel's struma found in the American literature are in Ewing's "Neoplastic Diseases," Crotti's "Thyroid and Thymus" and Marine's monograph on the thyroid in "Endocrinology and Metabolism."[1]

Among the outstanding features of this disease may be mentioned first the hardness of the thyroid mass, compared to iron, cartilage, bone and wood by various authors. The size has varied from that of an apricot to the mass shown in Fig. 1. Some authors, Spannaus, Delore and Alamartine, have claimed that goitre always precedes Riedel's struma but this is denied by Heinike. According to the reported observations the disease may appear in previously normal glands, in diffusely enlarged thyroids or in adenomatous nodules within the thyroid.

Riedel in 1896 and again in 1911 advocated partial resection. Ricard in 1901 removed the total mass for the first time. Ewing, 1922, advocates partial resection and radiation. Retrogression after partial extirpation has been a striking phenomenon of this disease and recovery after operation has been the rule. The administration of iodine has been without effect.

Post-operative myxedema, partial paralysis and operative death have occurred. In general the results of the more radical operations, such as attempted complete resection in the face of extensive adhesions, have been poor.

The general features of the reported cases are summarized briefly in Table 1.

The histologic findings in all cases agree for the most part in their essential details with those described by Riedel. The later authors have described the microscopic picture more minutely. A few authors, Murray, Hashimoto and Meyer, have found peculiar variations in structure but all agree that the condition is essentially a chronic inflammation.

[1] To these may be added the recent paper by A. W. St. George "Chronic Productive Thyroiditis." Annals of Surgery, July, 1924.

TABLE 2. *Reported Observations of Post-branchial Bodies in Human Subjects*

No.	Author	Year	Sex	Age	Condition	Thyroid	Post-branchial remnants			Location in thyroid, lobe
							Parenchyma	Accessory cysts	Main cyst	
1	Hermann and Verdun	1899	F	55 mos.	Embryo	Normal	+	+	–	Right
2	Hermann and Verdun	1899	M	63 mos.	Embryo	Normal	+	+	+	Both
3	Hermann and Verdun	1899	M	95 mos.	Embryo	Normal	+	+	+	Both
4	Getzowa	1907	M	56 yrs.	Cretin	Atrophic	+	+	+	Both
5	Getzowa	1907	M	47 yrs.	Cretin	Atrophic	+	–	–	Left
6	Getzowa	1907	M	56 yrs.	Cretin	Atrophic	+*	–	–	Both
7	Getzowa	1907	M	50 yrs.	Idiot	Atrophic	+	+ left	+ left	Both
8	Getzowa	1911	F	3 wks.	—	Athyriosis	+	+	+	?
9	Getzowa	1911	M	New-born	—	Normal	+	+	+	?
10	Getzowa	1911	?	90 mos.	Embryo	Normal	+	+ right	+ right	Both
11	Meeker	1923	M	50 yrs.	—	Riedel's struma	+	+	–	Both

* A benign tumor nodule developed from the post-branchial body in this instance.

In attempting to elucidate the peculiar pathology of Riedel's struma various authors have related it to Basedow's disease. Others have compared it to Riedel's pancreatitis, to Mikulicz's lacrymal granuloma, to Kuettner's disease of the salivary gland and to contracted kidney. To such a comparison there has been raised the objection that these glands possess open excretory ducts while the thyroid does not.

The presence of remnants of the ultimo-branchial body in the present instance is of peculiar interest, for should such remnants prove to be an essential feature of Riedel's struma, their presence would serve to relate this disease to the chronic inflammations of the glands just mentioned. Furthermore the peculiar pathology and rare incidence of the disease might then be explained as a result of extension of inflammation from the pharynx or trachea along the ultimo-branchial duct system, or its accompanying lymphatics, into the thyroid gland, an extremely rare phenomenon because this rudimentary duct system rarely persists even at birth (Table 2).

Summary

1. Riedel's struma is a chronic inflammation of relatively rare occurrence in the thyroid gland. It presents clinical manifestations very similar to those of malignant neoplasms in this organ, but is distinguished by its benign course when the pressure is relieved.

2. Recovery may be expected after partial extirpation or radiation. Iodine feeding is not beneficial. Total extirpation is frequently followed by myxedema.

3. Anatomically the disease is characterized in its early stage by hyperplasia of the thyroid parenchyma and later by an infiltration by lymphocytes, plasma cells and eosinophilic leucocytes with slight inflammatory manifestations on the part of the blood vessels. True lymph follicles are abundant in the early stages. As a result there is extensive degeneration of the parenchyma and proliferation of fibrous tissue which is remarkably abundant in the late stages. The inflammation may extend far beyond the limits of the gland.

4. The tonsils and peritonsillar tissue show a similar chronic inflammation and emphasize the pharyngeal involvement. The adjacent lymph nodes show chronic inflammation.

5. A specific causative agent has not been recognized.

6. Regenerative activity on the part of the parenchyma and persistent cell nests and accessory cysts representing the post-branchial body are observed for the first time in this case.

7. From these findings it is not unreasonable to assume that a primary pharyngitis or tracheitis may extend by way of the post-branchial system into the thyroid.

8. Since post-branchial remnants are found in human embryos, in athyriosis and in cretins and have not been found in normal adult thyroid glands, it is suggested that thyroids containing such rudiments may be of low vitality and quickly reach the stage of exhaustion and may, therefore, be peculiarly susceptible to the extreme form of atrophy and fibrous replacement seen in Riedel's struma.

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DESCRIPTION OF PLATES V–X

Fig. 1. Photograph of gross specimen.

Fig. 2. Low-power photomicrograph of a typical area. There is extensive fibrosis and diffuse infiltration by lymphocytes and plasma cells; a lymph follicle near the bottom.

Fig. 3. Higher magnification of an area showing lobule of thyroid gland. (1) Thyroid vesicles. (2) Lymphocytes invading vesicles and actually replacing the degenerated epithelium. (3) Lymph follicle.

Fig. 4. High-power drawing showing the fate of the thyroid epithelium. (1) Vesicle with colloid. (2) Degenerating vesicle. (3) Epithelial remnants of vesicle.

Fig. 5. Field adjacent to that shown in Fig. 4. (1) Epithelial remnants of thyroid vesicles. (2) Plasma cells. (3) Small lymphocytes.

Fig. 6. Another field adjacent to that shown in Fig. 4. (1) Thyroid epithelium. (2) Epithelial cell in mitosis.

Fig. 7. High-power drawing. (1) An epithelial cell nest representing the post-branchial body. (2) Remnants of thyroid epithelium. The epithelial nest is within a lymph follicle.

Fig. 8. High-power drawing of a group of solid cell nests similar to that shown in Fig. 7, found under the anterior capsule in lateral lobe. (1) Branching end buds of the parenchyma of the post-branchial body. (2) Cell nest with lumen lined by cuboidal cells.

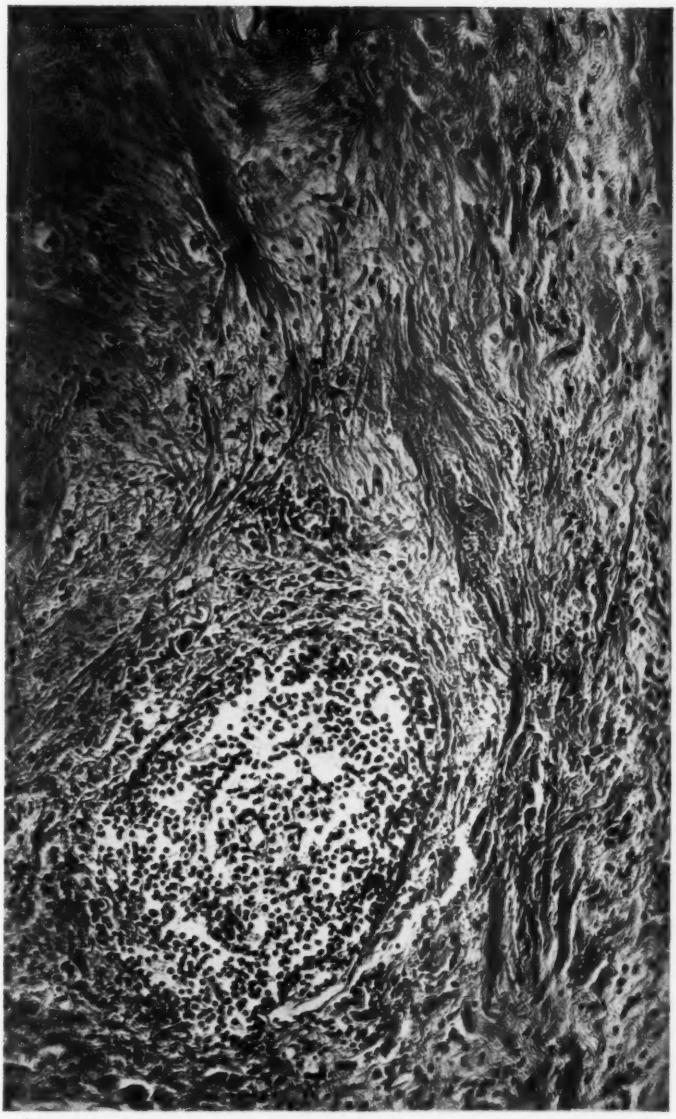
Fig. 9. High-power drawing of a group of epithelial cells representing an accessory cyst of the post-branchial body. (1) Wall of cyst. (2) Cavity of cyst. (3) Duct of cyst.

Fig. 10. Illustration (from Getzowa's paper) of an accessory cyst for comparison with Fig. 9. (1) Wall of cyst. (2) Cavity and duct of cyst. (*After Getzowa.*)

1

Meeker

Riedel's struma



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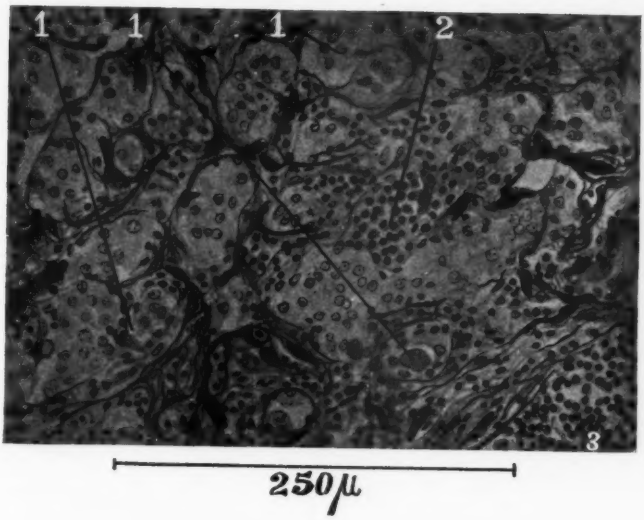
Meeker Riedel's struma



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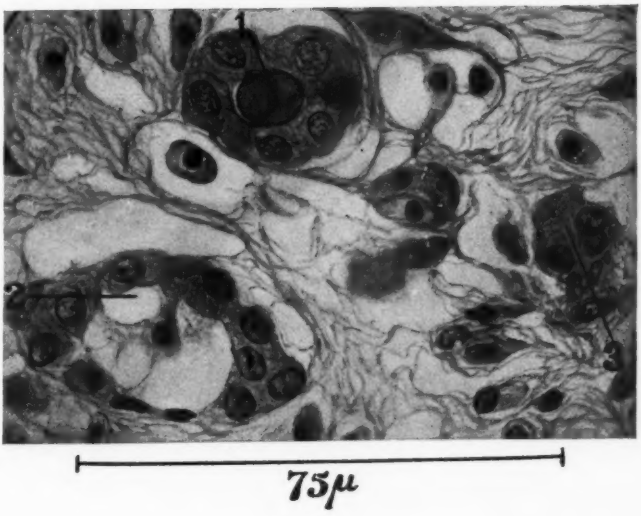

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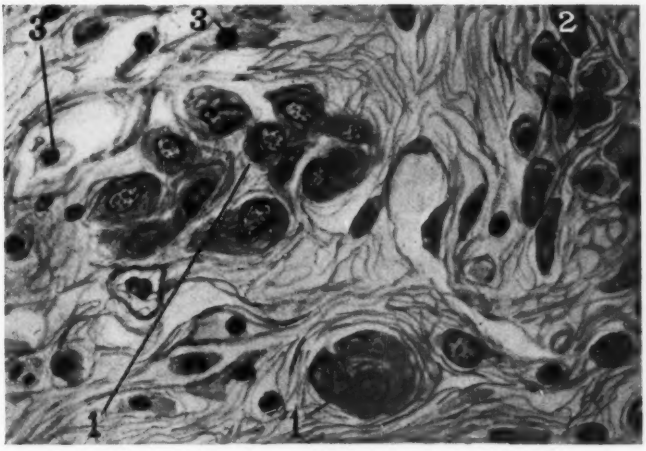
Meeker Riedel's struma



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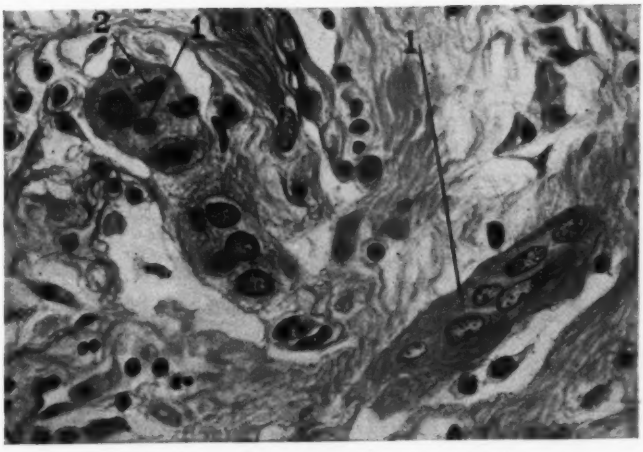

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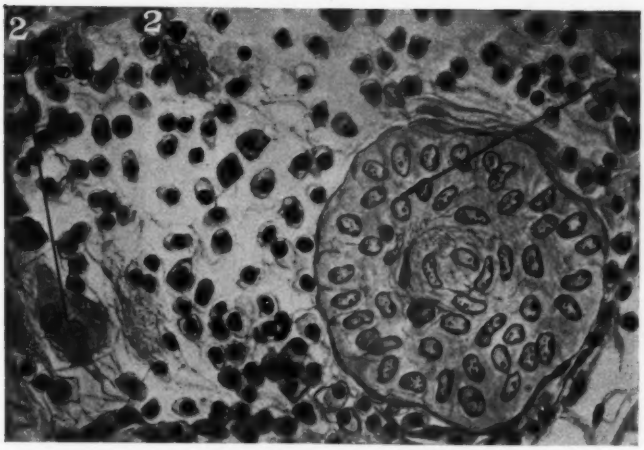

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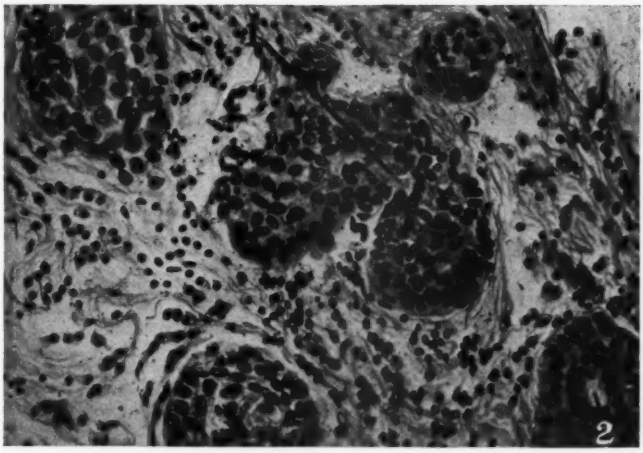

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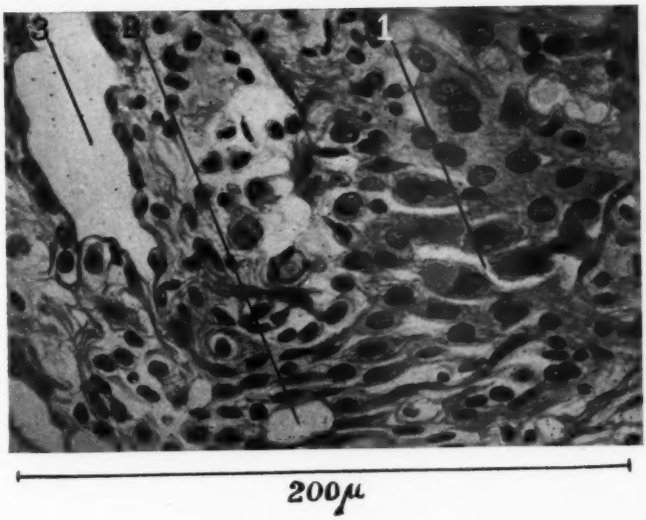
Meeker Riedel's struma



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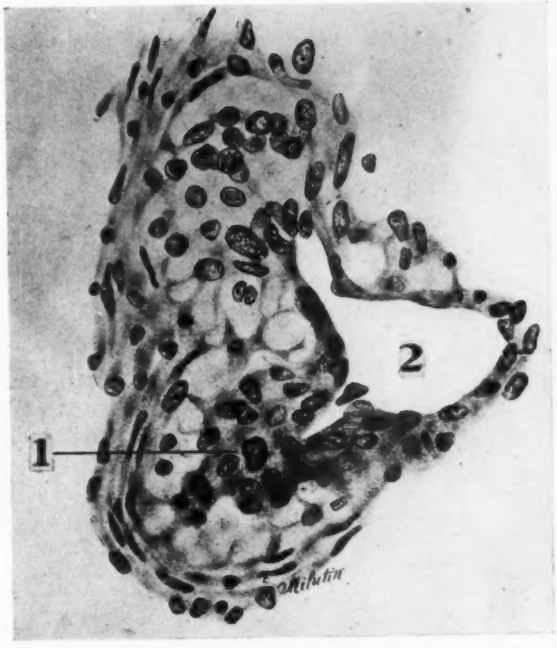

10

Meeker Riedel's struma

HETEROTOPIA OF THE BONE MARROW WITHOUT APPARENT CAUSE *

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The occurrence of tissue resembling bone marrow in positions other than in the bony cavities is uncommon and usually is found associated with bone, as a result of chronic inflammation, or appearing in some other organ of the hematopoietic system, as the result of a need for more blood cells in the circulation. The present case, however, is much rarer in that apparently normal (slightly hyperplastic) marrow was found in bilaterally symmetrical nodules, unassociated with bone, in a case in which the blood cell forming mechanism was apparently adequate.

Definitions

To clarify the meaning of certain terms used in this article, the following terms (which I have not been able to find grouped together elsewhere) are defined in accordance with accepted authorities.

Metaplasia is "The postnatal production of specialized tissues from cells which normally produce tissues of other orders and is an adaptation on the part of the cells to an altered environment" . . . "Yet metaplasia is bounded by rigid laws; epithelial tissue can only be converted into other forms of epithelial tissue, mesoblastic tissue only into forms of mesoblastic" (Adami and McCrae).[1]

In *heteroplasia*, "There is no conversion of one type of tissue into another, but there is merely a persistence of characters and cell relationship peculiar to an earlier period of growth."

Heterotopia "May be congenital or acquired and consists of the abnormal snaring of cells or organs from the organ proper, and then subsequent growth in another place." In the present article there is no implication intended as to how the tissue occurred in its abnormal site.

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Ectopia, though originally used more or less synonymously with heterotopia, is better reserved to apply to the misplacement of organs, usually congenitally, to neighboring regions.

Undifferentiation ("histological accommodation" of Aschoff) [2] is the loss of differential characters by cells which have become subject to abnormal conditions, for example, the flattening of cylindrical cyst epithelium through mechanical pressure.

Autochthonous growth occurs when cells, or tissue, abnormal in location are derived from cells formed in situ.

In contradistinction, *colonization* is the growth of tissue in an abnormal environment, originated by cells derived from distant points.

Heterotopia of the Bone Marrow

True bone and bone marrow are not infrequently associated together in metaplastic bone formation. This phenomenon, which usually is due to a long standing, chronic inflammation, has been found in the lungs, pleura, Fallopian tubes, kidneys, arteries and lymph nodes. An instance of such an occurrence in the lungs, which is the most common site of this phenomenon, was recently reported by Dr. M. Strumia from the Philadelphia General Hospital. The frequent occurrence of bone and bone marrow metaplasia in the hilum of the kidney is of interest in connection with Jordan's[3] recent report that this is one of the chief sites of blood formation in the frog's larva.

Bone marrow with normal cellular structure (i.e., with arteries, veins, fat and reticulum, as well as with the various appropriate blood cells) has rarely been noted in other parts of the body than the medullary cavities of the bone without association with bone. Some cases illustrating such a development of bone marrow without bone are given below, without touching on the rather extensive literature of myeloid metaplasia of the spleen (see Tanaka [4]). As a member of the hematopoietic system it is natural that the spleen should more easily react to this activity. Excepting in this organ I could find reference to only five cases in which it was not associated with either leukemia or pseudoleukemic anemia, though the literature on the subject was carefully searched.

In 1905 Gierke [5] reported a case in which bone marrow structure was found in the adrenal. The important associated lesions were

cardiac with hemorrhagic infarcts in the lung. He stated that Heidelberg had noted a somewhat similar case before and his was the second one of its kind.

In 1909 M'Kenzie, Browning and Dunn [6] recorded two cases in which they found typical bone marrow structure in the hilum of each kidney. The first case, aged 9 months, had massive confluent bronchopneumonia and a leukocyte count of 90,000 with many erythroblasts and other abnormal cells in the blood. The other case, 20 months old, had severe rickets. In the former case the bone marrow masses were present in the hilum around the vessels with some myelocytic proliferation throughout the whole kidney substance. The spleen and lymphatic glands were enlarged and showed myelogenic changes; the bone marrow showed evidence of active proliferation. In the rickets case the bone marrow masses were in the hilum and the kidney substance was not infiltrated. The bone marrow in the ribs was almost entirely replaced by osteoid tissue and fibrotic proliferation. The spleen was enlarged and showed evidence of myelogenic activity.

In 1912 Tanaka [4] reported two cases in which the bone marrow tissue was present in the hilum and substance of the kidney. One was 9 months old and the other 2 years old. Both of them had "splenic anemia" and severe rickets.

In 1918 Matsunaga [7] and in 1919 Mieremet [8] each reported a case of heterotopia of the bone marrow. In the former it was present in the hilum of the kidney, the patient having had myelogenous leukemia with a leukocyte count of 144,000. In the latter case it was present in the adrenal, the patient having had carcinoma of the esophagus and terminal aspiration bronchopneumonia.

In 1922 Herzenberg [9] described three cases. In two the heterotopic bone marrow was present in the pelvis of the kidney; in both instances the patient was 2 years of age and had splenic anemia. In the third case it was found in an accessory adrenal, in a patient aged 69 years who died from cardiovascular disease.

CASE REPORT

The case the author wishes to present apparently differs in several respects from any hitherto recorded. So far as can be determined by carefully reviewing the literature and by personal communication with various pathologists (Drs. A. J. Smith, McFarland, H. Fox,

Ewing, Krumbhaar, Sabin and Lucke) it is the first one of its kind noted. The protocol in brief is as follows:

Clinical History: L. B., white female, aged 81 years, admitted to the Philadelphia General Hospital Feb. 18, 1924.

Family History, Negative.

Past History: Nothing of importance and nothing suggesting anemia.

Present Illness: She had an attack of hemiplegia 2 months ago and has been in bed ever since. Four weeks later she became irrational.

Physical examination revealed an obese white female with senile dementia, right hemiplegia, general arteriosclerosis, and hypertrophied heart with a systolic murmur; death occurred one week after admission from terminal bronchopneumonia.

Necropsy notes: Adult white female, height 155 cm., weight 135 lbs., autopsy performed 23 hours after death.

Heart, hypertrophied, weighing 570 gms.

Arteries, general arteriosclerosis.

Lungs, patches of bronchopneumonia.

Spleen enlarged, weighing 405 gms.

Liver, congested; gall bladder, filled with gall stones.

Kidneys and other organs showed arteriosclerosis.

Brain showed arteriosclerosis, multiple areas of thrombotic softening, pituitary adenoma.

On each side of the thorax at the fifth rib near the vertebral column a small, reddish, smooth, encapsulated, rounded and nodular mass was noted. Each one measured 2.5 cm. in diameter (Fig. 1). They were adherent to the ribs with fibrous tissue. After their removal the ribs beneath were smooth and showed no erosion or disturbance of the periosteum. No connection between them and the marrow in the medullary cavities could be determined. No similar masses were found anywhere else in the body. No bony disturbance was noted, although, as the true nature of these tumors was not suspected, no examination of the bone marrow was made. The cut surfaces of the two masses were alike, purplish red, moist, smooth, elastic and with a homogeneous appearance. The capsule was fibrous and thin. The whole mass looked like an organizing blood clot.

Microscopically several sections from each mass showed slightly hyperplastic normal bone marrow structure with some hemorrhage. The structure consisted of wide capillaries and between them the parenchymal cells — Myeloblasts, myelocytes, leukocytic eosinophiles, eosinophilic myelocytes, multinucleated giant cells, erythrocytes and erythroblasts, many fat cells, connective tissue cells and blood vessels occurring approximately in the proportion normally found in hyperplastic marrow (Figs. 2 and 3). There was much pigment both within the macrophages and outside them, especially at the margins of the tissue (Fig. 3). Sections from the various areas gave much the same picture. The oxydase stain confirmed the presence of the myelocytic cells (Fig. 5). The iron stain gave a positive Prussian blue reaction, showing that the pigment originated from the red cells.

DISCUSSION

This case apparently differs from any other reported in which bone marrow heterotopia has been found both in the location and symmetrical arrangement of the masses, and in their separation from any of the organs of the body; also it differs from most cases in not having any apparent cause. In the bone metaplasia cases, for instance, each was accompanied, or preceded, by an inflammatory process. In the other cases the heterotopic bone marrow structure was present in pathological conditions of the blood-forming organs except Mieremet's case, carcinoma of the esophagus, M'Kenzie's, Browning's and Dunn's case, severe rickets, two mentioned by Gierke, and Herzenberg's third case, cardiovascular disease. In these cases the adrenals, and the pelvis and the hilum of the kidney were the sites of this phenomenon and in all the hematopoietic system was more or less involved.

Why is this heterotopic bone marrow most commonly found in the adrenal and the hilum of the kidney? M'Kenzie, Browning and Dunn stated that examinations of fetuses 10 weeks to 9 months old showed, lying in the hilum of the kidney around the vessels, small masses of tissue which give evidence of hematopoietic activity. And in very early embryonic life these masses are situated in close relationship to the cortical layer of the adrenal. Maximoff is said to have produced experimentally bone and bone marrow in the pelvis of the kidney by ligating the vessels. Thus the location of bone and bone marrow in this region suggests either a persisting embryonic function, or a renewed activity due to some unknown stimulus, of a function which ceased after birth.

The hemorrhage and deposits of blood pigment, both striking features in all the sections, are probably due to the imperfect circulation in the heterotopic tissue which caused stagnation of the red cells after their formation.

The reason for the occurrence of heterotopic bone marrow is still in dispute. Two theories are offered: One group of investigators believes it is autochthonous, while others believe it is formed by colonization by emboli derived from the blood forming organs. According to Herzenberg the former theory is supported by Sternberg, Schridde and Maximoff; the latter by Ziegler and Ribbert. Tanaka considered the heterotopic bone marrow in the two cases he

reported to be of embolic origin. Pollack,[10] who with Lubarsch made an extensive study of myeloid metaplasia in the lymph nodes, believes in the autochthonous theory. Ewing [11] in discussing the heteroplastic deposits in leukemia seems to be in favor of the embolic origin. MacCallum [12] after discussing the origin of the myeloid foci in the distant organs says, "To me the idea of transplantation of cells seems more plausible."

Gierke, who found heterotopic bone marrow in the adrenal, believed this to be metaplasia from local cells, and considered it tumor formation having normal bone marrow elements. These cells he thought were brought there during embryonic life and later developed.

Mieremet and Herzenberg agree with Gierke and consider their cases to be of autochthonous origin from congenitally misplaced cells.

Those that support the colonization theory are principally the authors who found the bone marrow tissue in the pelvis and the hilum of the kidney in cases of leukemia and pseudoleukemic anemia. On the other hand those that support the autochthonous theory are the authors who report heterotopic bone marrow tissue in the adrenals. They consider the misplacement of the bone marrow cells to have occurred in the embryo with the migration of the sympathetic tissue to form the medullary structure.

In the case under consideration it is hard to conceive a way in which the colonization could have occurred, because so far as is known no disease of the blood forming organs was present. Therefore it more likely originated autochthonously by metaplasia or developed from congenitally misplaced bone marrow cells. In view of the symmetrical arrangement the latter is more probable. The majority of those with whom I have discussed this case support this view, while Dr. Ewing, who does not believe they are of embolic nature, thinks it is compensatory effort on the part of nature to supply the necessary amount of blood cells. But it is hard to believe it compensatory, even though the rest of the bone marrow was not examined, in view of the absence of any evidence of a demand for an excess of blood cells. No one to whom it has been shown thinks it is a tumor in the sense of an ordinary myeloma.

It is necessary, however, to consider the possibility of the true neoplastic nature of these tumors. If this is granted, a type of

benign myeloma must be conceded in which all the elements of the bone marrow are present and in approximately normal proportions.

An interesting comparison with this case is here suggested by the finding in another case recently studied in these laboratories (antemortem report by Young and Cooperman [13]). In a case of osteitis fibrosa cystica autopsied in June, 1922 (No. 6824), the typical bony changes of this disease were found in the pelvis, humerus, vertebrae, ribs, etc. In the thoracic cavity arising from the parietal pleura a large rounded mass, reddish and measuring about 6 cms. in diameter, was noted. Though adherent to lung substance with fleshy bands and spicules of bone, it had probably arisen from the rib. The microscopical sections, which were taken several centimeters away from the rib, showed a typical hyperplastic bone marrow structure containing the various cellular elements previously mentioned, with the exception of the giant cells which were a predominant feature in the sections of the bone marrow proper. This histological difference, together with the extensive destruction of bone marrow through the body suggests that in this case, we are dealing with a compensatory effort (metaplasia from connective tissue), though it is not possible to rule out that this may be a myeloma of mixed cell type having a metaplastic origin. If this is considered to have arisen in adult life from misplaced bone marrow anlage, it could properly be considered a neoplasm, though essentially differing from the previous explanation only in the time element; if the development occurred in embryo then the previous explanation still holds.

SUMMARY

1. A case is reported, apparently unique, in which two symmetrical masses of slightly hyperplastic bone marrow were found attached to inner surface of the ribs in an old female hemiplegic, dying of terminal bronchopneumonia.

2. Various theories as to their etiology are considered. The autochthonous origin from heterotopic bone marrow cells or multipotent connective tissue cells, during embryonic life is thought more probable, but the possibility of their being benign bone marrow tumors must be considered.

3. Examples of myeloid metaplasia and bone marrow heterotopia from the literature are also considered, as well as the statement that

bone marrow structure is always found around the vessels in the hilum of the kidney and in the cortex of the adrenal in embryonic life.

4. A comparison is made with somewhat similar tissue, found in a case of osteitis fibrosa cystica.

The author wishes to express his appreciation to Dr. Krumbhaar for his help in preparing this paper.

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DESCRIPTION OF PLATES XI–XII

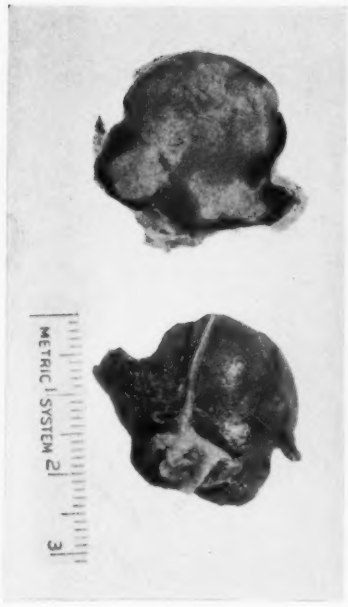
Fig. 1. Heterotopic bone marrow mass, external and cut surfaces.

Fig. 2. Histology of mass–hyperplastic bone marrow showing primordial cells; myelocytes, eosinophilic myelocytes, polymorphonuclear leukocytes, normoblasts, erythrocytes, megakaryocyte, fat cells. X 230.

Fig. 3. High power view showing leucogenetic and erythrogenetic centers. X 736.

Fig. 4. Margin of mass, showing macrophages, loaded with hemosiderin pigment. X 184.

Fig. 5. Goodpasture stain, showing oxydase granules in many of the cells. X 920.



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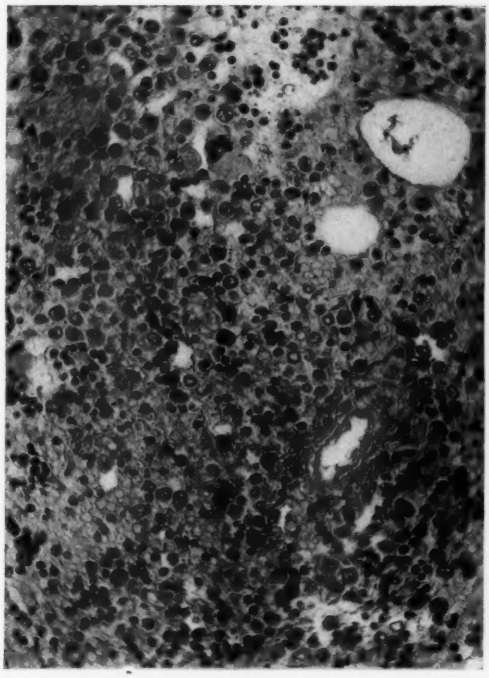

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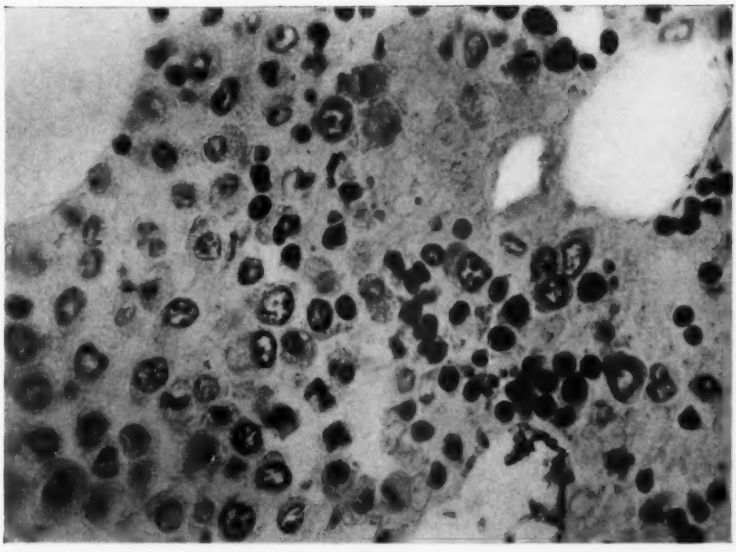
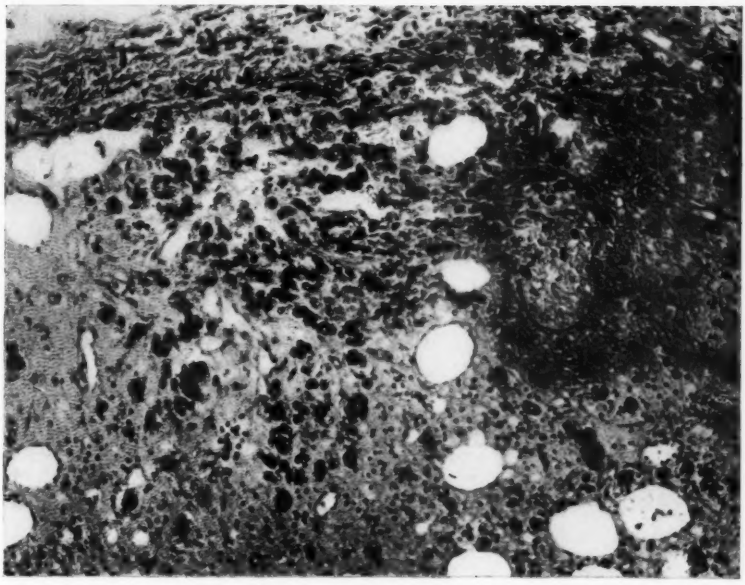


3

Saleeby

Heterotopia of the bone marrow



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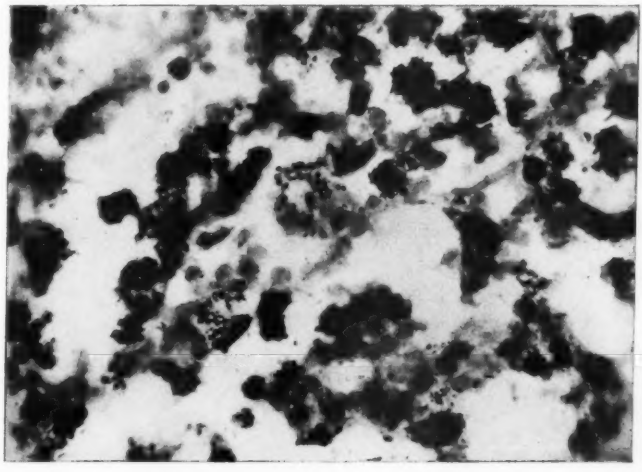

5

Saleeby Heterotopia of the bone marrow

MICROGLIA AND THE PROCESS OF PHAGOCYTOSIS IN GLIOMAS*

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Recognition of a cell group in the central nervous system, distinct from neuron, neuroglia, and connective tissue, is an event of importance. Because of the nature of its well defined function, the activity of *microglia* is of particular importance in pathological conditions. Some of the stages in the activity of these cells in such conditions have long been recognized and bear familiar names: "Nissl's Stäbchenzellen," "ameboid wandering cells," "Gitterzellen," "granulo-adipose cells," "scavenger cells," etc.

There is a large group of cells, without previously recognized expansions, which are scattered everywhere throughout the central nervous system, and which have been called "apolar," "indifferent" or "adentritic cells," "naked nuclei" or "satellite cells," and "third element of Cajal".

Del Rio-Hortega has demonstrated that these "naked nuclei" are in reality possessed of complicated cytoplasmic prolongations and further, that we are dealing not with one class of cells but with two distinctly different groups.[22] One group, mesoglia of Robertson,[25] is considered by Rio-Hortega to be of ectodermal origin. He therefore prefers the name "neuroglia of few expansions" or "*oligodendroglia*" [17, 18].† The second subdivision, described by Rio-Hortega, has been called by him *microglia*.[19, 20]

* Received for publication September 4, 1924.

Some of the microscopical work was done in the Department of Pathology, Presbyterian Hospital.

† There has been some confusion on this point. Robertson, in a brief note, described a group of cells stained selectively by his platinum method. Without reporting any inquiry into their histogenesis, he assumed them to be of mesodermal origin. Because of the structure of these cells, and the fact that they are very numerous and appear frequently in rows between the fibers of the white matter, there can be no doubt that they are identical with oligodendroglia. These cells are probably of ectodermal origin (as maintained by Rio-Hortega) but final judgment on this point must await further histogenetic study.

The mesodermal origin and the function of microglia cells, on the other hand, has been described at some length and will be discussed briefly in this paper. Cajal, in

We have studied this element in human material and that of cats, rabbits and mice. It is present in both the white and gray matter of the brain and spinal cord. The nuclei of these cells are typically small and usually elongated. When stained by Nissl's method, they are seen to contain heavy chromatin granules. The cytoplasm and expansions can be impregnated by silver carbonate. Under normal conditions, microglia cells present many long attenuated expansions, quite irregular in shape and giving rise to numerous smaller branches and spines (Fig. 1 A). The shape of these prolongations depends somewhat on the pattern of the surrounding structure, as the microglial processes insinuate themselves into the interstices of the neuron warp.

The development of microglia is of considerable interest and throws light on its pathological changes. The idea of immigration into the central nervous system of mesodermal cells is a very old one. Thus Boll [4] in 1874 described movable cells in embryos which he believed to be neuroglia of mesodermal origin. The presence, in the brains of new-born infants, of granular ameboid cells, sometimes containing fat, led Virchow [28] to attribute their presence to "encephalitis intersticialis neonatorum," as early as 1867. These cells were variously considered to be normal and having to do with myelinization or to indicate an abnormal inflammatory process.

The cells described by these earlier investigators (complete reference is not attempted here) correspond to newly formed microglia, as demonstrated by the method of Rio-Hortega. These cells are few in number in the embryo until about the time of birth when there is a very rapid increase. This increase continues for the first few weeks following birth, after which time there is little change in normal mammals.

There are two chief "fountains" of microglia or places where the cells are first seen in large numbers, i.e., (i) the tissue beneath the ventricular ependyma at the site of the invagination of the pia to form the tela choroidea, (ii) the under surface of the cerebral peduncles. In these areas microglia cells are seen in large numbers immediately beneath the pia. From here there is a rapid spread of the cells along the clefts of the white matter. Thus, in the early days

1920, although accepting the establishment of microglia as an entity, was unable to satisfy himself that the rest of the adentritic or third element cells could be made to correspond to Rio-Hortega's description of oligodendroglia.

after birth, all of the microglia is in the white matter and none is to be seen in the grey matter, while after the first or second month the number in a field of grey matter is usually larger than in an equal field of white matter. The cytoplasm of the new-formed cells is finely reticular and contains small clear vesicles, or later vacuoles. During the stage of what appears to be migration, they take on various ameboid shapes with the frequent formation of pseudopodia. The transition from these migration forms to the fully ramified adult form can be seen with great clarity in brains (e.g., of rabbits) from two to ten days of age.

The author has never been able to see the actual transition from pial fibroblast to microglia cell as the method seems to stain cells only when they take on the aspect of microglia. Probably it is only when the mesodermal cells, which lie next to the brain, begin to ingest cerebral substances that they are rendered stainable by silver carbonate. Consequently, a layer of flattened microglia cells is found between the under surface of the pia and the nervous tissue. These flattened cells are not interspersed with larger ones engorged by phagocytosis. Such cells lying about blood vessels will be described below. The *possibility* that microglia may be derived as well from the adventitia of the larger cerebral vessels, about which they congregate, must be considered; also, the possibility of derivation from embryonal or polyblastic cells, whose existence has been urged by many investigators, cannot be denied.

In pathological conditions involving a destruction of the cerebral tissue, e.g., about areas of softening, there is a rapid and easily demonstrable reversion of the microglia in the neighborhood to ameboid forms (Rio-Hortega,[20, 21, 22]). That is, the complex expansions become less ramified and the cytoplasm approaches its nucleus. The cell migrates to the site of the lesion and takes on the well known characteristics of Gitterzellen (Abräumzellen, granulo-adipose cells). Collado [8] has described somewhat similar changes of microglia in cases of rabies.

Metz and Spatz, in a recent paper,[13] confirm the morphological character of microglia ("Hortegaschen Zellen") described above and the conversion of this element into "Gitterzellen." But they believe these cells to be fixed and non-migratory. They continue to agree with the German investigators who preceded them that Gitterzellen may likewise be formed by neuroglia and fibroblastic cells. If the

phagocytes formed by these last two types of cell possess the power of movement, as they admit, it is difficult to understand how the microglia, in its transformation to Gitterzellen, can remain a fixed element in pathological conditions. They incline to the view that microglia is not of mesodermal origin but is a type of neuroglia. Nevertheless, there is in their most interesting paper a new proof of the functional differentiation of these two types of cell; i.e., that in general paralysis iron pigment is found in large amounts in microglia while none is to be seen in neuroglia cells (nor, for that matter, in nerve cells).

In experimental brain wounds of short duration, studied by the author, the change in the form of microglia cells is so well graduated at different distances from the lesion as to leave no doubt that microglia develops *phagocytic* and also *migratory* power, thus surrounding the lesion in a very short time with a cluster of granulo-adipose cells (see also Rio-Hortega[21, 23]). These early sections contain no evidence of a similar metamorphosis on the part of other types of cells. It is, of course, impossible on these grounds to maintain that granulo-adipose cells can never be derived from other types of cells under different conditions.

Finally, it must be admitted that the actual transition stage from fibroblast to microglia has not yet been demonstrated. Nevertheless, because of the nature of microglia reaction to pathological conditions, its resemblance to macrophages seen in other parts of the body, the absence of any transition between it and neuroglia, and, most of all, because of the location and time of appearance of the "fountains of microglia" as well as its manner of invasion into the central nervous system, microglia must be considered of mesodermal origin. It forms, therefore, a third element in the central nervous system.

Case I* is that of a patient (A.R.) in the Presbyterian Hospital, New York City. Briefly, the history was of more than a year's duration, the chief characteristics being gradual mental change with headaches and several attacks of unconsciousness. At operation, the author turned down a large bone-flap exposing an extremely tense dura. A needle, passed through the dura into the left frontal lobe, yielded a small amount of clear yellow fluid. The bone-flap was

* The presence of microglia in a number of gliomas not reported here was established, although the conditions prevented perfect staining results.

therefore closed at once with a tentative diagnosis of cystic glioma. The patient died six days later and autopsy showed a large infiltrating tumor which involved both frontal lobes and contained two small cysts, one of which was filled with blood (Fig. 2).

Over a year later blocks were taken from the tumor and stained by Rio-Hortega's silver carbonate method for microglia.* As judged by these sections and the routine sections made at the time of autopsy, the tumor is a glioma. It contains a moderate number of nerve cells (cf. 27, 16). The neoplastic cells grow close together in some areas and in a lax formation in others. Occasionally they are arranged in small rings, calling to mind the structure of the ependyma. (Stroebe,[27] Mallory,[12] Bonome,[5] Ranke,[16] Spiller, Bailey.[2])

Microglia in various stages of migratory and phagocytic activity can be seen everywhere throughout the tumor. By the silver carbonate method these cells and the fibers and cytoplasm of giant neuroglia cells are stained selectively, while only the nuclear outlines of the neoplastic cells are visible. With this method it will frequently be found that only the pathological neuroglia is stained.

These giant cells (Fig. 3) contain large irregular nuclei which are often multiple or in process of amitotic division (cf. Achucarro [1]). In some cases daughter nuclei appear to be leaving the cytoplasm (Fig. 3, A and B, see also Borst [6]). These cells elaborate long well-formed fibers which take the silver stain energetically (Fig. 4) and pass out a considerable distance through the neoplastic parenchyma, providing the greater part of the fibers visible in the tumor. Stroebe, in 1895, and Ranke, in 1911, among others, suggested that most of the fibers seen in gliomas are produced by previously existing neuroglia as a reaction to the presence of the tumor. The cytoplasm of these cells is large in amount and at times contains vacuoles shown by Mallory's stain (Fig. 3), or granules when impregnated with silver (Figs. 5 and 6). This change in cytoplasm indicates degeneration and is accompanied by fragmentation and disappearance of the prolongations and by gradual loss of the cell outlines (Fig. 7).

Such regressive change in neuroglia cells was taken by Rosental [26] to be preliminary to an ameboid wandering stage, a transformation

* The author did not succeed in interpreting these sections until he had later studied the development and behavior of microglia under the generous personal guidance of Dr. del Rio-Hortega, who also called to his attention the fact that no study has been previously reported of the relation of microglia to gliomas.

he reported in various pathological conditions of the brain. His illustrations show clearly first, degeneration of neuroglia cells similar to that described above, and second, smaller cells said to be ameboid glia but which resemble ameboid forms of microglia. Held's migratory cells, described in normal brains, may have been of a similar nature. Likewise, Alzheimer believed in a regressive transition from neuroglia to "Gitterzellen." Bonome [5] was of the opinion that the granulo-adipose cells found in gliomas were largely of fibroblastic origin, arising from the vascular adventitia, but he also believed a small proportion of these macrophages were derived from the regression of giant neuroglia cells.

In an excellent paper written in 1914, Ziveri [29] maintained that the above change in neuroglia was analogous to degeneration of nerve cells and that the cellular changes were of similar character. Although he had not at his command methods for staining the intermediary stages, he urged that it was the third element of Cajal which had to do with the catabolic changes in the central nervous system and not neuroglia. With a modification of Alzheimer's method, he described giant neuroglia with fragmenting expansions and about these cells grandulo-adipose cells. Unfortunately, no conclusions can be drawn from his illustrations, but he is of the opinion that the function of the third element is *similar* to that of granulo-adipose cells.

Whether or not the giant neuroglia cells in the tumor under consideration arose from the tumor itself or were originally proper to the brain structure is only a matter of conjecture. Certain it is that these fibrous neuroglia cells resemble at certain stages the giant glia seen in other pathological conditions of the brain.

There is no evidence that these degenerating forms take upon themselves phagocytic activity.* The changes are *purely degenerative* in character. With fragmentation of the fibers (Fig. 6), the cytoplasm becomes filled with argentophile granules and vacuoles which may eventually be represented only by fine dust (Fig. 7). The cell outlines become progressively fainter and eventually indistinguishable. The cause of this degeneration is not apparent. It

* Cajal (Trab. d. Lab. Invest. Biol. XI, '13, bottom of p. 297) noted hyperplasia of the neuroglia about experimental brain wounds and the evidence of phagocytosis on the part of third element cells but found no indication of transition from one type of cell to the other.

does not seem to be due directly to lack of blood supply in the neighborhood of the cells themselves, for such changes are seen in cells which are close enough to functioning vessels to implant vascular feet on their walls (Penfield [14]).

The rôle played by microglia in this process of neuroglia degeneration is of considerable interest. In early stages microglia cells are found in relation with the *neuroglial processes*, showing a predilection for smaller processes. Each cell applies itself to a fiber or engulfs its termination in greedy cytoplasm (Fig. 5, A, B, C). Later, as the prolongations begin to break off, the macrophages approach more closely and envelop fragments (Fig. 6, A, B, C), or absorb the stumps of large prolongations (D, E, F). There comes a time, however, when the stumps of the dismantled cell no longer attract microglia (Fig. 6, G, and Fig. 7). At no time was phagocytosis of the cell body seen.

During digestion of the fiber fragments, microglia cells appear to pass through several characteristic phases. When a cell first becomes applied to a fragment it usually contains a somewhat oval nucleus at the end of an elongated bag-shaped protoplasm. The nucleus is unstained and the cytoplasm faintly impregnated and possessed of very fine scattered granules, most noticeable at the cell surface. Argentophile granules appear about the nucleus, especially at one pole (Fig. 8, A). The cytoplasm of these cells may take any shape, however, and the uniformity of outline is due to the constant shape of the subjects of phagocytosis. Where more than one fiber is being engulfed as B, C, D (in Fig. 8), this form varies according to the situation. The argentophile granules become larger first near the nucleus and later in the rest of the cell body (Fig. 8, E). These granules, or, better, globules, become numerous and the cell assumes a more or less spherical form (Fig. 8, F, G). Such forms are usually found near vessels. So much is this the case that one is forced to assume migration of the cell at this stage to the vessel. Cells H and K with unstained large vacuoles, no doubt represent some phase in fiber digestion.

In some regions microglia, scattered through the neoplasm, seem to be almost exclusively devoted to phagocytosis of neuroglia processes. There are other areas where the microglia cells, for the most part spherical, crowd closely and are filled with colorless vacuoles. These typical "Gitterzellen" indicate destruction of cerebral tissue.

Often the intercellular spaces in such areas contain argentophile droplets and these the macrophages ingest (Fig. 9, A, B, C).

Certain areas, which contain no evident products for phagocytosis, present many microglia cells whose ameboid shape suggests migration (tuberous and pseudopodic forms of Rio-Hortega) (Fig. 9, E). These shapes tend to be parallel as though the direction of the migration were common to all.

There are likewise areas of microglia proliferation where mitotic figures are plentiful. The tissue of these areas is sufficiently lax to allow the cells to take on rounded forms and many free argentophile droplets are present. The various forms drawn in Fig. 10 were all found within a small compass. Some cell outlines were very indistinct, others easily visualized. No division by amitosis was encountered.

Examination of the blood vessels gives ground for certain interesting suppositions concerning the activity of microglia cells. In the vicinity of vessels is to be seen a large proportion of cells heavily laden with granules. Only the refractile outline of the vascular wall can be seen. Microglia cells are found plastered over the outer surface of vessels in the tumor. Various stages in the reduction of cytoplasmic contents and size can be seen in Fig. 11. The appearance of cells A and B suggests new arrivals heavily laden, C to G, stages in delivery of the cytoplasmic contents, and M and N, cells almost ready for renewed phagocytosis.

Such close relationship of granulo-adipose cells to the vessel wall has been taken by many investigators to mean that these cells arise from *adventitial* fibroblasts. The possibility of such origin in some conditions cannot be categorically denied, but Rio-Hortega's specific method enables one to see all stages of these cells with a clarity denied to previous observers. The constant phases illustrated in Fig. 11 suggest a delivery of cell contents, perhaps by osmosis, through the cell wall into the vessel (or perhaps into the perivascular space). If this is the case, one microglia cell may be many times liberated for its proper activity as a scavenger.

Forms O and P (Fig. 11) were seen in the relation illustrated, about various vessels. They are microglia without cytoplasmic inclusions and therefore ready for phagocytosis. They resemble closely cells in the first stage of phagocytosis of fiber fragments described

above (Fig. 8 A). The transition from heavily laden cells to these smaller forms is uninterrupted and without evidence of new formation. The conclusion, in this case at least, is that microglia cells migrate to the vessels, deliver their cytoplasmic contents through the vessel walls or into the perivascular space and return to a *renewed phagocytic activity*. Contributory evidence toward the truth of such a supposition is provided by the active mitotic increase in microglia cells which was seen to be independent of blood vessels (Fig. 10). It is also of interest to point out just here that Metz and Spatz [13] have shown that in general paralysis iron pigment is found exclusively in microglia *and* cells of the vessel wall.

Case II is that of a child of 8 years in the Hospital Clinico de la Facultad de Medicina, Madrid. From the age of seven she had complained of failing vision, followed by blindness, convergent squint, headaches and vomiting. Shortly after admission to the hospital the patient died and autopsy showed a tumor of the roof of the midbrain, extreme hydrocephalus and "enormous swelling of the optic nerves."

Blocks of the tumor were sent by Prof. Suner to Dr. del Rio-Hortega, to both of whom I am indebted for the opportunity to study the material. The neoplasm is a glioma. Its cells appear fibrous in places and are closely packed. In other areas the structure is more lax. Ependymal ring formation is to be seen occasionally. Fibers of neuroglia type are found to be plentiful when stained by the silver carbonate method for neuroglia. There are areas of marked softening in the tumor.

When stained by Rio-Hortega's method for microglia, these cells (microglia) are found to be plentiful in zones encircling the foci of tumor softening. Figure 12 shows such an area of degeneration (A). Vessels in this focus and at its border are thrombosed (H). The zone between neoplasm (N) and softening (S) is occupied by microglia cells in active *dendrophagocytosis*. The neuroglia fibers in the zone are evidently degenerating as they take the stain intensely and are being rapidly devoured. No microglia cells were found in well vascularized areas of tumor nor within the focus of softening.

In this case, as in the first case reported, the activity of microglia within the neoplasm is restricted to dendrophagocytosis. It seems therefore that microglia cells subserve two principal functions in re-

lation to gliomas, i.e., phagocytosis of degenerating neuroglia fibers within the tumor and phagocytosis of the products of cerebral destruction in the brain surrounding the neoplasm.

Large phagocytic cells have been recognized in tumors of various types in other tissues. They have been considered as derivatives of one or more of the following cell forms: fibroblasts, vascular endothelial cells, leucocytes, and ubiquitous undifferentiated polyblastic cells. In a recent publication,[24] Rio-Hortega and Asua have described fully the structure of these cells. They call attention to the striking morphological similarity between the above macrophages and the phagocytic cells of the central nervous system (ameboid forms of microglia).

Conclusion

The activity of microglia in the brain surrounding a glioma is similar to that described by Rio-Hortega in other destructive processes of the central nervous system. These microglia cells act as scavengers to clear away the products of degeneration and cerebral destruction.

Giant neuroglia cells in the neoplasm undergo degeneration with fragmentation and loss of their processes. This change is *purely degenerative* and is not a stage in the formation of ameboid phagocytes. The supposition that such a change was possible has probably been due to confusion of this degenerated cell with phagocytic microglia. That neuroglia cells ever metamorphose into microglia appears to be extremely improbable.

In the nervous tissue surrounding the tumor microglia cells take on ameboid forms. The relation of these cells to the degenerating neuroglia within the neoplasm is a constant one, consisting of phagocytosis of the prolongations (dendrophagocytosis) but not of the cell body. In areas of cerebral softening the microglia cells crowd closely, assuming the more spherical and reticulated form typical of "Gitterzellen." They resemble the macrophages of fibroblastic origin seen in various neoplasms, as described by Rio-Hortega and Asua.

Regions of very active mitotic division are to be seen. This cellular reproduction does not involve the intervention of either neuroglial or adventitial cells.

Microglia cells seem to transport the ingested substances to the

outer surface of blood vessels where they lose their granular and vacuolar appearance, decrease in size, and finally leave the vicinity of the vessel in characteristic form for renewed phagocytosis. The inference is made that the digested contents of these cells pass into the vessel lumen or into the perivascular space. The presence of scavenger cells about vessels indicates transfer and delivery of ingested substance rather than new formation of these cells from fibroblasts.

Microglia seems to discharge two chief functions with relation to a cerebral glioma: (*a*) within the tumor, dendrophagocytosis and (*b*) in the nervous tissue surrounding the tumor, phagocytosis of the products of cerebral destruction.

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DESCRIPTION OF PLATES XIII–XX

Fig. 1. Transition from normal microglia cell A to granulo-adipose cell E and F, characterized by shortening and swelling of expansions, swelling of cell body and vesiculation of cytoplasm. Cell A was taken from another case, as it is almost impossible to stain a normal cell completely after long fixation in formol, although pathological forms still stain. B to F are from brain adjacent to the tumor. All preparations illustrated were made by the Silver Carbonate Method of Rio-Hortega unless otherwise stated.

Fig. 2. Cross section of brain showing location and extent of tumor.

Fig. 3. Giant cells from tumor stained by Mallory's Phospho-tungstic Acid Hematoxylin Method. A, B, and C degenerating cells with vacuolar cytoplasm and nuclei which have undergone simple division; D, radiating partition of nucleus; M, nuclei of microglia; N, same with cytoplasm faintly stained.

Fig. 4. Two giant neuroglia cells. Microglia scattered among the faintly stained neoplastic nuclei.

Fig. 5. Giant neuroglia cell in early degeneration. The cytoplasm is granular and microglia cells A, B, C have applied themselves to its expansions at their terminations.

Fig. 6. Giant neuroglia cell in advanced degeneration. A, B, and C, microglia ingesting fiber fragments. D, E, and F, microglia applied to stumps of the remaining expansions. Expansion G, as well as the cell body, apparently does not attract phagocytes.

Fig. 7. Final stage in degeneration of neuroglial cells.

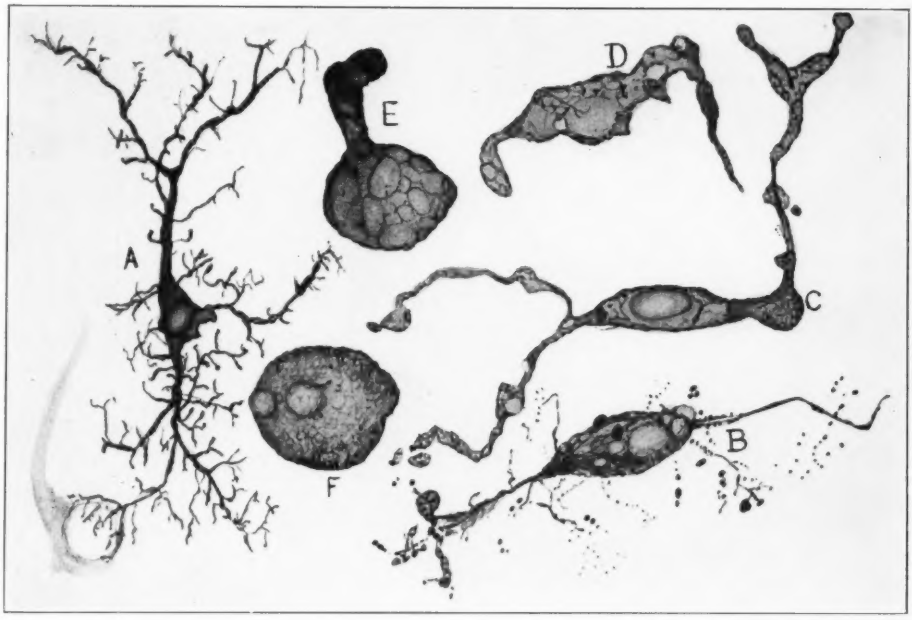
Fig. 8. Stages of ingestion of glial fiber fragments by microglia, illustrating the manner in which the cytoplasm follows the fiber form. A, earliest stage, to G, final stage. H and K are stages in fiber digestion.

Fig. 9. Ameboid phagocytic microglia ("Gitterzellen," "granulo-adipose cells").

Fig. 10. Mitotic division of microglia.

Fig. 11. Blood vessel in glioma surrounded by microglia in various stages of delivery of ingested substance (A to G). M to P, emptied cells.

Fig. 12. Area of softening (S) within glioma, case II, surrounded by a zone of microglia in active dendrophagocytosis (B). H, thrombosed vessels. N, neoplasm.



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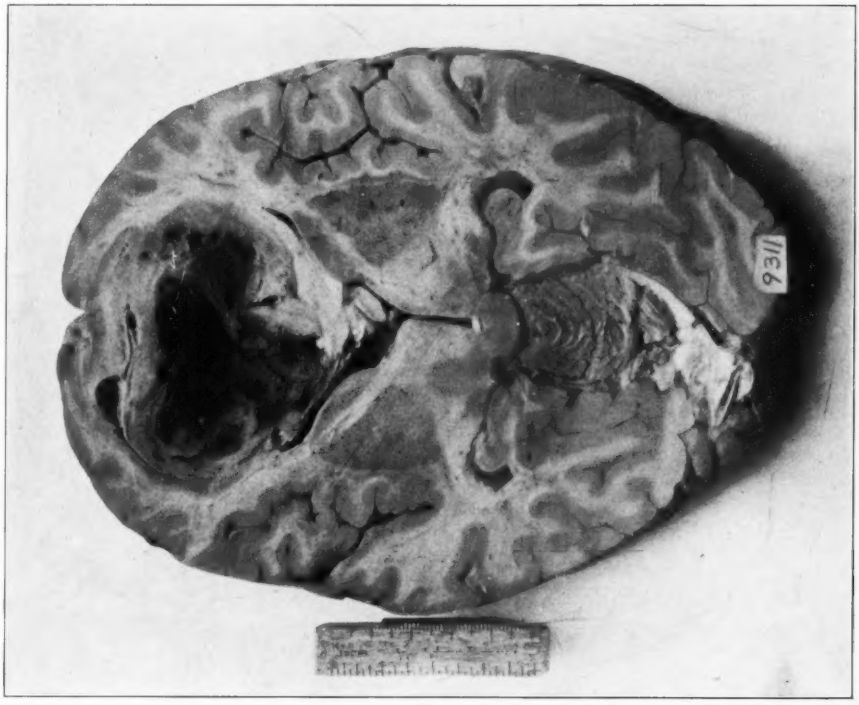

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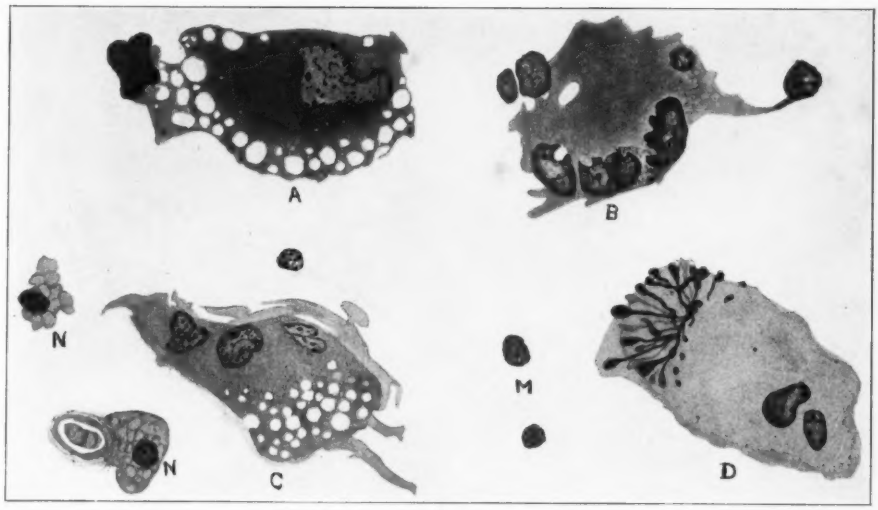

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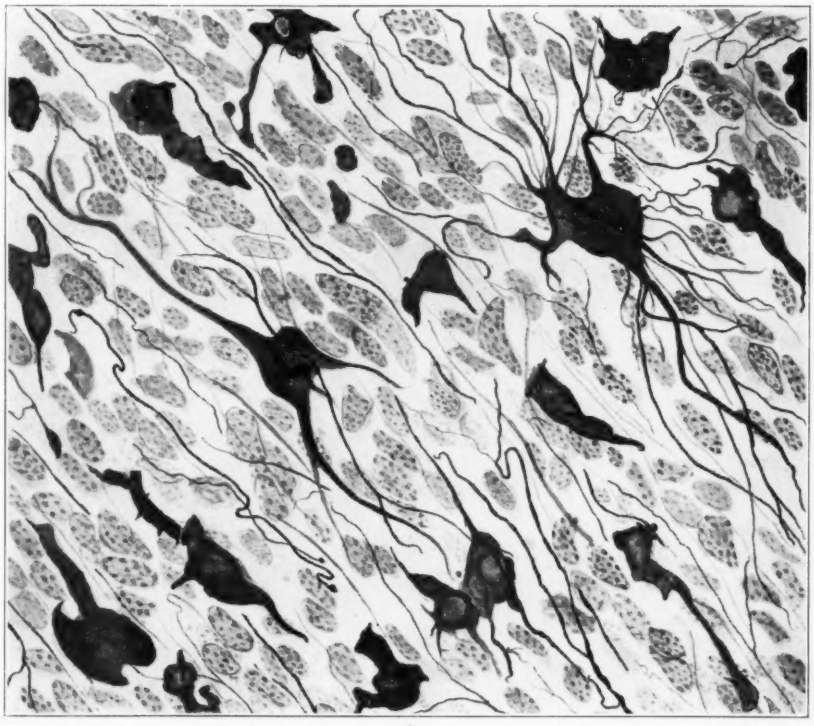

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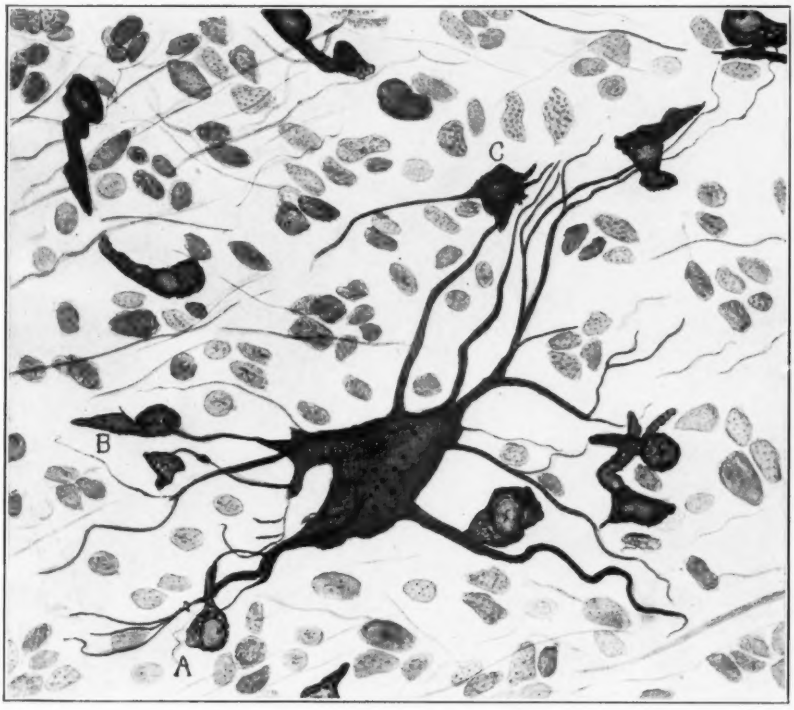
Penfield Microglia



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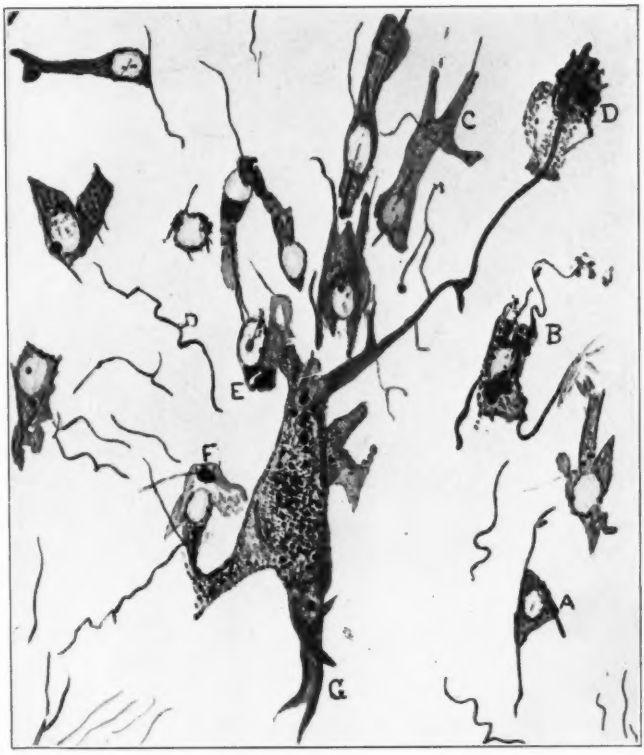

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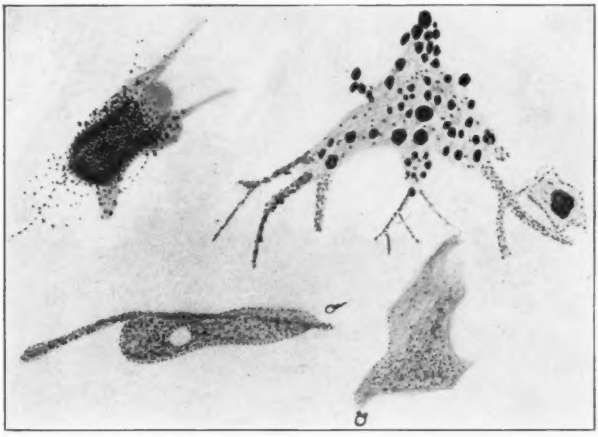

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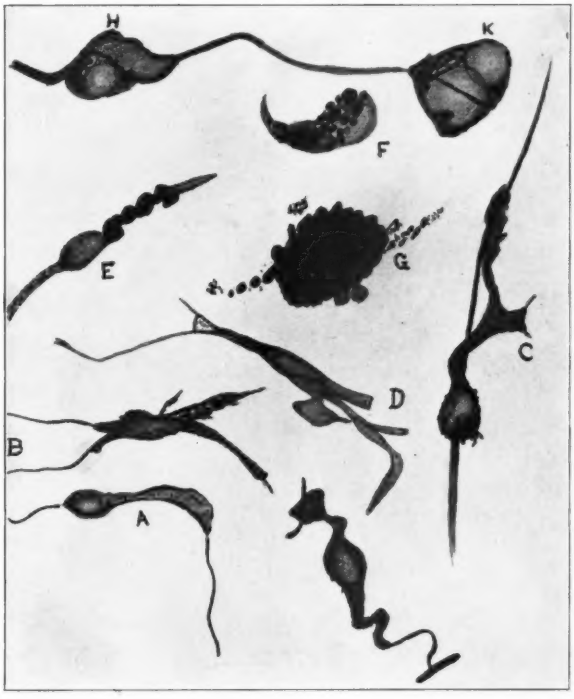

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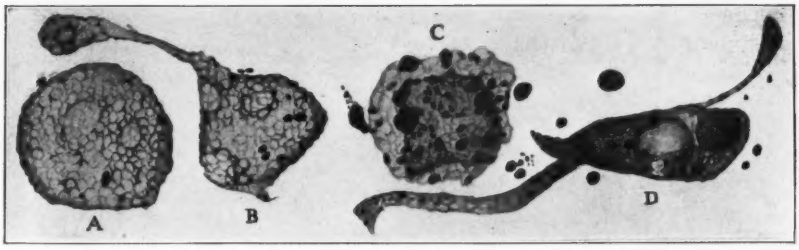

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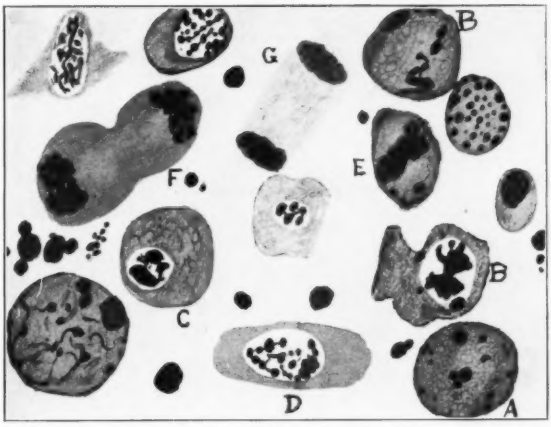

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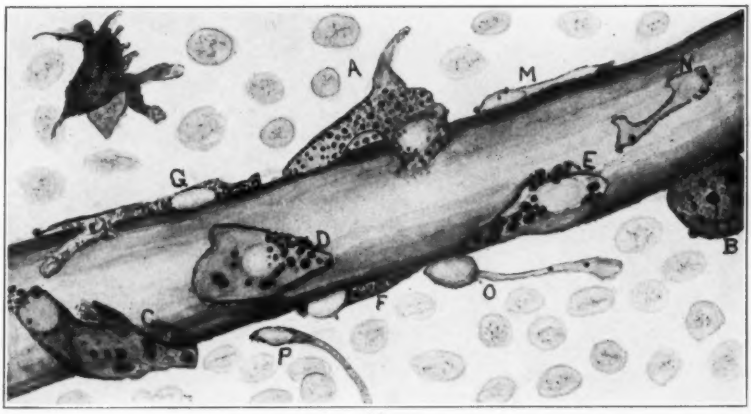

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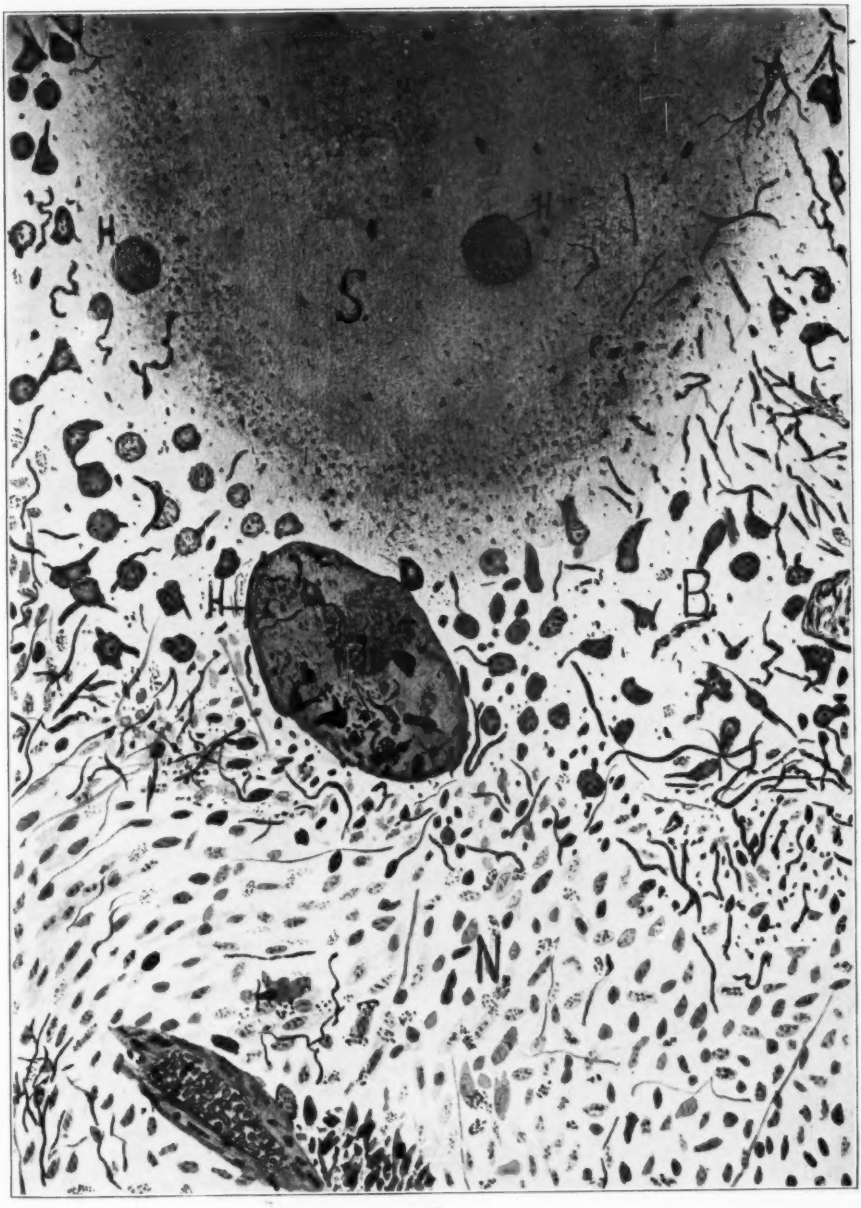

12

Penfield Microglia

THE FORMATION OF MACROPHAGES, EPITHELIOID CELLS AND GIANT CELLS FROM LEUCOCYTES IN INCUBATED BLOOD *

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While tissue cultures would seem to afford an appropriate technic for the study of the part taken by the white blood-cells in various conditions, this method has seldom been employed. In 1914 Awrorow and Timofejewskij studied the white blood-cells of leukemic blood in plasma cultures, Loeb (1920) followed the amebocytes of the king crab in clotted blood, and Carrel (1921) observed the growth of the buffy coat of the centrifuged blood of the adult chicken in plasma cultures. The method by which the observations incorporated in this paper were made is simpler than that of any of the above investigators, consisting merely of the incubation of hanging drops of blood taken, by means of a paraffined pipette, either from the heart or from the peripheral circulation.

The transformation and growth of the leucocytes into macrophages, epithelioid cells, and giant cells were observed in the blood of the chick embryo, young chicken, adult hen, mouse, guinea-pig and dog, and in human blood. In every kind of blood examined there developed first a large wandering cell, several times larger than any of the normal leucocytes, which was phagocytic for red blood-cells, melanin granules, carbon particles, dead granulocytes, and tubercle bacilli. Somewhat later there appeared a cell more like a primitive mesenchyme cell, and still later the epithelioid cell was formed. This cell was sometimes binucleate and in some instances a typical multinucleated giant cell (Langhans giant cell) was formed. Since the transformation and growth of the leucocytes were much the same in the different bloods examined, the details of the phenomenon in avian and human blood only will be described.

Avian Blood. The transformed cells occurred in incubated blood taken from the adult fowl as well as from embryos of various ages, the youngest used being 6 days' incubation. Usually the blood

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studied was taken from chicks just hatched or just about to hatch, but several series were from chicks one week after hatching, and two were from young chickens two months old.

When the blood culture was studied within an hour or so after preparation, it had a number of granulocytes with large, usually spindle-shaped granules, and some non-granular cells (Fig. 1) migrating about on the coverglass. At this time these two types of leucocytes showed the usual difference in size, and all of them contained typical leucocytic nuclei. Within a few hours one or two refractive globules appeared in some of the non-granular cells (Fig. 2), which were then considerably larger than the granular cells. After twelve hours there were always several cells much larger than normal. These hypertrophied cells contained no specific granules, but usually a few refractive globules (Fig. 3). They grew in size and also divided, increasing in number so rapidly that often after two or three days the culture contained hundreds of them (1200 were counted in one drop) and only a few granulocytes. As the cells hypertrophied there was a coincident change in the nucleus, which lost its compact character and became larger, paler, and showed definite nucleoli (Fig. 4). After 48 hours, while most of them were from three to four times as large as the granulocytes, not only in length and width but also in thickness, smaller ones were still present (Fig. 5), some no larger than the original leucocyte. The cells now contained a number of refractive globules of different sizes, some quite large, most of which stained with Sudan III, while some appeared to be plasma granules. Such globules are not peculiar to the transformed leucocytes, as fat globules and plasma granules accumulate in most tissue cells grown in drawn blood or plasma. The cultures favorable for observation were those in which the cells did not form many of these globules.

After 24 to 48 hours the transformed cells were very phagocytic and took up various kinds of foreign bodies, but they seemed especially phagocytic for the red blood-cells of their own blood and most of them contained one or more erythrocytes, some of which were partly digested (Fig. 6). As many as twenty such cells were seen in a single macrophage. The ingested blood-cell soon died, as was indicated in preparations stained with neutral red by the fact that when first taken in the red blood-cell did not take the stain, but soon afterwards the nucleus became red and later the whole erythrocyte

became slightly red. Fragments of red blood-cells were also ingested and these stained red. In many instances the ingested cell crumpled up; in others it became laked, so that when stained it appeared as a fluid vacuole containing a red body — the nucleus. In later stages of digestion the nucleus of the ingested cell remained for some time after the rest of the cell had disappeared (Fig. 7). The macrophages resembled the clasmatocytes of the chick connective tissue to such an extent that it was difficult to distinguish the two cells from each other when placed side by side. The transformed leucocyte was usually somewhat larger and contained larger fat globules, otherwise each cell had the eccentrically placed nucleus, the thick cytoplasm, and the delicate, sheet-like processes continually changing shape and position. When stained with neutral red they each contained many red bodies scattered irregularly throughout the cytoplasm.

Some cells, migrating on the coverglass, were separated by a layer of serum from the red blood-cells so that they ingested few or none of the latter. These cells spread out on the glass, sometimes long and slender and joined end to end, or large and flat with a centrosphere. While these cells seldom contained ingested material they were nevertheless phagocytic; if the drop was shaken so that the erythrocytes came in touch with them, ingestion often followed and, as was observed in a few instances, the cells drew in their cytoplasm, became loosened from the glass, formed processes and became wandering cells. The clasmatocyte-like cells also occasionally became attached to the coverglass where they spread out into large flat cells in which a centrosphere was sometimes evident.

After the blood had been incubated 2 or 3 days some of the large flat cells became transformed into "epithelioid cells" (Fig. 10). By this term is meant, not all cells that resemble an epithelial cell, for that would include practically all of the large flat cells, but a specific large cell containing, around the centrosphere, a peculiar large central area which stains as a finely granular red area when neutral red is placed upon the preparation. This cell has a large nucleus with definite nucleoli usually eccentrically placed at one side of the central area. Beyond the central area, or just in its outer edges, there may be seen particles of ingested material and usually, in tissue cultures, many small fat globules in this outer region. Beyond this, the cytoplasm is extended out into an exceedingly delicate

peripheral film, the outer limits of which are almost impossible to distinguish in the living preparation, but which often become curled and drawn in, especially upon fixation, giving the characteristic curled edge so often seen in permanent preparations. Lewis and Webster (1921) described this cell in cultures of the human lymph node; Maximow (1924) observed the same type of cell in cultures inoculated with tubercle bacilli, and I have frequently obtained an abundant growth of this type of cell in cultures of isolated tubercles. The epithelioid cell developed earlier in chick blood than in the mammalian blood. The central area was neither so large nor so marked as in that of human blood, but larger than the centrosphere and stained in the characteristic manner.

Some of the transformed leucocytes became multinucleated. When this occurred, coincident with becoming epithelioid cells or later, the resulting giant cells were of the Langhans type. They contained from two to ten nuclei and were usually larger than the same type of cell containing only one nucleus, but not always so. The giant cells sometimes contained ingested material (Fig. 9) and in a few instances were observed to ingest red blood-cells, even after they contained several nuclei, but usually these cells digested what foreign bodies they contained and did not ingest any more.

The transformed leucocytes of the chick seldom lived more than seven to ten days in the incubated drops of blood, but if removed to plasma drops they lived for three or four weeks. It was in such preparations especially that the wandering cells became indistinguishable from clasmatocytes of the connective tissue subjected to the same environment. When plasma cultures of transformed leucocytes were incubated with avian tubercle bacilli the cells ingested large numbers of the latter. They digested the bacilli and behaved in every way as did the clasmatocytes when exposed to these organisms (Smith, Willis, and Lewis, 1922). In one such culture, fixed and stained after 20 days' incubation, there were observed, side by side, an elongated fibroblast-like cell (Fig. 12), a round epithelioid cell with a large central area (Fig. 11), and a clasmatocyte-like cell (Fig. 8), each containing tubercle bacilli.

When trypan blue or pyrrol blue was introduced into the culture, the granulocytes did not form blue granules; but after 48 hours the hypertrophied cells contained many, depending upon how much they came in contact with the stain.

Mammalian Blood. It was a simple matter to obtain an abundant growth of the transformed leucocytes of the chick's blood and practically every drop incubated contained many of them; but it was more difficult to get the same results with incubated drops of mammalian blood, and for this reason the following method was sometimes used as a control. Ten cubic centimeters of blood were rapidly drawn into a paraffined tube by means of an oiled cannula and centrifuged for about five minutes, most of the plasma withdrawn and the tube incubated. The buffy coat became a thick, tough layer of cells, extending up into the plasma and down among the red blood-cells. In this layer, after two to four days' incubation, the transformed leucocytes were abundant; many of them contained ingested and partly digested red blood-cells and some were multinucleated giant cells. These cells remained alive in the tube a number of days and living wandering cells were found in the bottom of the tube (two inches under the surface) even after ten days' incubation.

In the incubated drops of the blood of the mouse the cells grew well, regardless of whether the blood was taken from the heart or from the peripheral circulation. They did not multiply so extensively as those from the chick, but became many times larger than the normal cells (compare figure 14 with figures 15, 16, 17). They were phagocytic, as is shown in figures 15 and 17. The nucleus became large and contained definite nucleoli. Most of them were macrophages; a few were of the epithelioid type, as shown in figure 16.

The hanging drops of human blood were made from blood taken from the finger. These were usually injured by contact with the glass, and while the cells often lived for two or three days and displayed the beginning of the transformation, they more frequently died before the formation of an epithelioid type of cell, unless the coverglass was coated with some substance more favorable for their development. The best results were obtained by coating the covers with celloidin. In some of these cultures of human blood the white blood-cells lived for twenty days, in a few they lived nearly four weeks.

The first change in these cells also was the formation of refractive globules, which occurred after twenty-four hours, but the cells hypertrophied much more slowly than did those of the chick, so that it was often as long as four or five days before many unusually large

cells were seen. These cells were phagocytic and contained many red blood-cells; they increased in number until the eighth or ninth day. Scattered refractive globules of different sizes were present within them (Fig. 21), but these were much smaller than the globules in the hypertrophied cells of the chick blood. The nucleus became more homogeneous with more definite nucleoli. About the seventh day a few large flat cells appeared migrating on the coverslip. They were triangular, round, or spindle-shaped, and while at first they sometimes showed the remains of one or two ingested red blood-cells (Fig. 22), they soon completely digested these and took in no more. This was probably due to the fact that they did not come in contact with any, for if the blood was stirred, or fresh blood added, they again ingested red blood-cells, often in great numbers (Fig. 23). Instead of a few scattered refractive globules of different sizes, they contained many very small ones (Fig. 24). After eight or nine days these cells had increased greatly in number and had the appearance of epithelioid cells, i.e., they were large and flat with a large pale nucleus containing definite nucleoli, a large central body surrounded by a layer of fat globules and debris, and a delicate peripheral film of cytoplasm. When the preparation was stained with neutral red the central body became stained red (Fig. 26). A few of these cells became multinucleated and formed typical giant cells, but this was by no means so frequent an occurrence as in the cells of avian blood.

From the ninth day on, the majority of the hypertrophied cells formed in the human blood were of this type, although there were always present a few migrating phagocytic cells, containing red blood-cells, and some long spindle-shaped cells (Fig. 25). They did not retain the same shape from day to day; a round cell was observed to change into a long, thin, spindle-shaped cell and then into a triangular cell. These changes took place gradually and the cells migrated very slowly. They continued to increase in number for several days, after which they increased slightly in size and died when between three and four weeks old.

Although the transformed cells of the human blood lived so much longer than those of the chick, they never multiplied to the extent that the latter did. In cultures of chick blood, after a few days of incubation there were hundreds of these cells, but the greatest number ever seen in a single drop of human blood was 360 in a culture that had been incubated ten days. Occasionally a cell hypertrophied

rapidly and even ingested red blood-cells before the nucleus had lost its leucocytic character.

In regard to the red blood-cells, it is impossible to state how long those of the mammalian blood can live; they often seemed to be in good condition for as long as six or eight days. Those of the chick, however, are nucleated and form vacuoles which stain with neutral red, so that it could be determined that many of these remained alive for as long as eight days, as was shown by the fact that the nucleus did not stain with neutral red, while the vacuoles did.

Discussion. Whether more than one type of blood-cell hypertrophies and gives rise to the transformed cell is difficult to state. Awrorow and Timofejewskij conclude that the lymphocyte is the stem-cell from which arises the enlarged mononuclear cell and from it develop the other types of transformed cells found in their plasma cultures; i.e., the wandering cell, spindle-shaped cell, phagocytic cell, giant cell, and the cell which these observers call the "Auslauferzelle." They hesitate to call the cells either clasmatocytes or fibroblasts and, although they have found cells resembling the clasmatocyte and others resembling the fibroblast, these authors decided that, on the whole, the majority of the cells are not entirely like either of these types of connective-tissue cells. Carrel states that the granulocytes disappeared from his cultures after a few passages, and since he failed to find the small mononuclear after the first week, he supposed that these cells either were transformed into the large mononuclear leucocyte or died out, while the large mononuclear cells proliferated, migrated and, under certain conditions, changed into cells resembling fibroblasts and into transition forms, half fibroblast and half ameboid cells. He also observed typical macrophages in his plasma cultures. Clark and Clark (1920, 1923) were able actually to follow the living cells throughout their activity in sterile inflammation in the tadpole's tail. These investigators state the polymorphonuclear leucocytes migrated out from the blood-vessels toward the site of inflammation, where they became stationary, with spherical nuclei, sent out processes, and came to resemble fibroblasts; and that as the inflammation subsided, these cells again became ameboid and wandered away. In my cultures of chick, mammalian, and human blood, granulocytes were not observed to become transformed, and in blood containing a great

many of them there did not occur a proportionately greater number of transformed cells, nor did the hypertrophied cells contain neutrophilic or eosinophilic granules. So far as could be determined by following the cells in the incubated drops of blood, it seemed to be the mononuclear type that gave rise to the three kinds of transformed cells, i.e., the macrophage, the epithelioid cell, and the giant cell.

Sabin (1921, 1923) and her co-workers, Cunningham and Doan (1924), have been carrying on a series of most interesting observations in which they differentiate the various types of blood-cells by means of vital dyes, and on this basis attempt to establish a grouping of the blood-cells more in accordance with what they consider to be their origin. By this method these investigators, in their later publications, distinguish the monocyte from the clasmatocyte and claim that, while both the monocyte and the clasmatocyte are phagocytic, there is nevertheless a distinct difference between the two types of cells. In view of this it is rather interesting that some of the transformed leucocytes certainly resemble clasmatocytes, whether the term be employed in its usual sense of tissue macrophage or in the more restricted meaning assigned to it by these later writers. These clasmatocyte-like cells may not be true clasmatocytes, but neither can they properly be termed monocytes, owing to their increased size, changed nucleus, accumulated fat globules, irregularly scattered ingested material and neutral-red bodies. Just what term should be used to designate these cells does not decrease the importance of the fact that macrophages, epithelioid cells and giant cells do arise from the leucocytes of the blood, without, in this case, any possibility of participation in the phenomenon by the endothelium or the connective tissue.

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DESCRIPTION OF PLATE XXI

Transformed leucocytes in the incubated drops of blood, drawn while living. Ocular No. 6, 2 mm. lens. [Reduced one-half in reproduction.]

Fig. 1. The normal cells of avian blood. These were drawn one hour after the cultures were prepared. Granulocyte, mononuclear, lymphocytes, and nucleated red blood-cell.

Fig. 2. Non-granular cell in blood from a 2-months' old chicken after 6 hours' incubation.

Fig. 3. Hypertrophied non-granular cell in blood from a 2-months' old chicken, incubated 12 hours.

Fig. 4. Transformed leucocyte containing fat globules and red blood-cells in same culture as that of Figs. 2 and 3, after 24 hours' incubation. The nucleus has lost its leucocytic character.

Fig. 5. Phagocytic cell containing a red blood-cell in same culture as that of Fig. 8. This cell had not hypertrophied.

Fig. 6. A phagocytic wandering cell containing many red blood-cells. From the blood of a week-old chicken, incubated 3 days.

Fig. 7. A phagocytic wandering cell spreading out on the coverglass. It contained laked red blood-cells and the nuclei of many digested ones. From the blood of a 2-months' old chick, incubated 4 days.

Fig. 8. A phagocytic wandering cell which was transferred to a drop of plasma containing avian tubercle bacilli. This cell ingested many organisms and lived for 20 days.

Fig. 9. A giant cell containing partially digested red blood-cells. Blood from a week-old chicken — after 4 days' incubation.

Fig. 10. An epithelioid cell in a drop of blood from a 19-day old chick embryo, after 3 days' incubation.

Fig. 11. An epithelioid cell containing many tubercle bacilli. From the same preparation as that of Fig. 8 — after 4 days in plasma.

Fig. 12. A long spindle-shaped cell containing ingested avian tubercle bacilli in same culture as that of Figs. 8 and 11.

Fig. 13. Red blood-cell from the blood of the mouse.

Fig. 14. Mononuclear cell from the blood of the mouse.

Fig. 15. Phagocytic cell from a drop of mouse blood incubated 40 hours.

Fig. 16. Round flat cell of the epithelioid-cell type from a drop of blood of the mouse, taken from the abdominal circulation and incubated 48 hours.

Fig. 17. Phagocytic cell from a drop of blood from the heart of the mouse, incubated 48 hours. It contained many red blood-cells, each of which was in a different stage of digestion.

Fig. 18. Mononuclear cell from human blood, one half hour after the culture was prepared.

Fig. 19. Polymorphonuclear cell from human blood, one half hour after the culture was prepared.

Fig. 20. Human red blood-cell, one half hour after the culture was prepared.

Fig. 21. Phagocytic wandering cell of the clasmatocyte type, containing ingested erythrocytes; drawn after 7 days' incubation of human blood on celloidin.

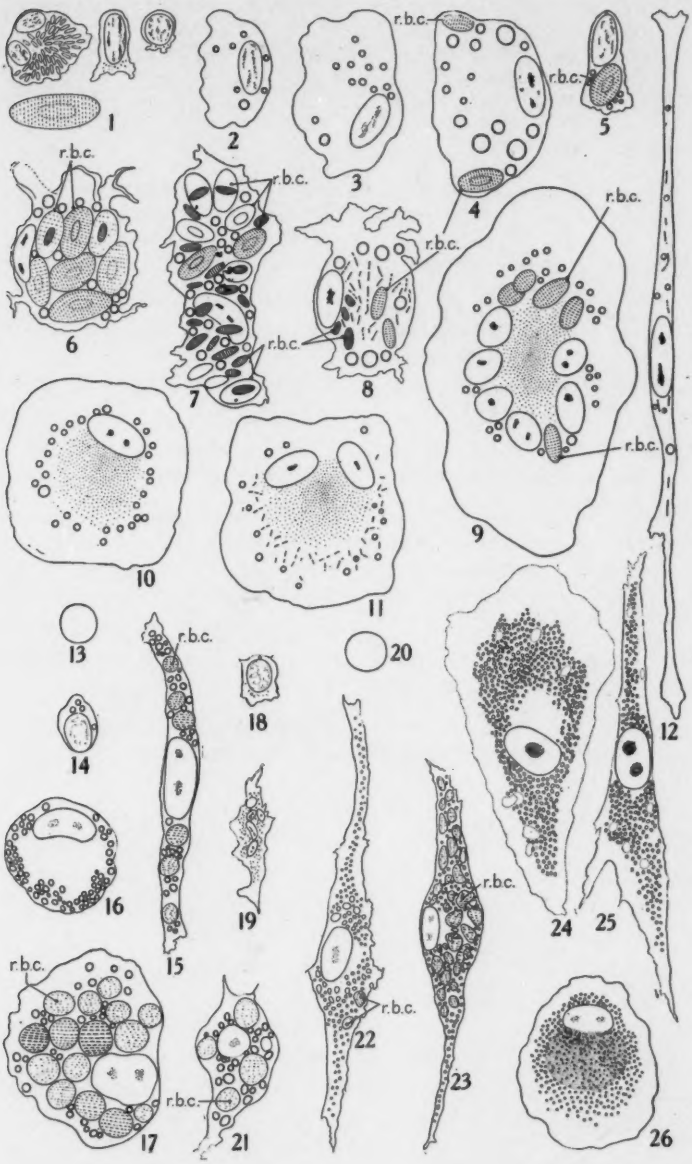
Fig. 22. A spindle-shaped cell from the human blood after 8 days' incubation.

Fig. 23. A cell of the fibroblast type, which, after it had grown eight days on celloidin, was again exposed to red blood-cells. It ingested many red blood-cells. These were partly laked and partly digested at the end of 10 days when the preparation was drawn.

Fig. 24. Epithelioid type of cell with enlarged centrosphere and large pale nucleus containing a nucleolus. Drawn after a drop of human blood had been incubated 13 days on celloidin.

Fig. 25. Spindle-shaped cell from human blood incubated 9 days.

Fig. 26. An epithelioid type of cell, showing the enlarged stained centrosphere; drawn from human blood, incubated 8 days on celloidin.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STUDIES ON BLOOD FIBRIN *

ITS QUANTITATIVE DETERMINATION; NORMAL FIBRIN VALUES, AND FACTORS WHICH INFLUENCE THE QUANTITY OF BLOOD FIBRIN

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Introduction. The origin of fibrinogen has been credited to various tissues of the body. Brown-Séquard,[1] Dastre,[2] Mathews [3] and others concluded that it was formed in the coats of the intestine. On the other hand, Doyon and Gautier [4] came to the conclusion that the intestine played no part whatever in the formation of fibrinogen. Goodpasture [5] decided that while the intestines were not essential to its production, they contributed to its formation. The lungs and the skin have also been considered seats of origin of fibrinogen.[2] Müller,[6] Morawitz and Rehn,[7] and others decided that it was formed in the bone marrow or by the leucocytes. This was supported by the high fibrinogen content of the blood in leucocytosis and in septic conditions, and by myeloid changes observed in the bone marrow, spleen, and liver following defibrination. So much for the extrahepatic origin of fibrinogen.

Of particular interest is the evidence which has been accumulated to show that the primary seat of fibrinogen production is the liver. In 1894 Corin and Ansiaux,[8] and a little later Jacoby,[9] showed that the incoagulability of the blood in phosphorus poisoning is due to a disappearance of the fibrinogen. These observations were later definitely associated by Doyon, Morel and Kareff [10] with retrograde changes in the liver. At the same time Doyon [11] showed that large doses of chloroform by mouth caused a marked drop in the fibrinogen. This was also associated with pronounced changes in the liver. A drop in the fibrinogen was also observed by Whipple and Hurwitz[12] following prolonged chloroform anesthesia which produces a fatty degeneration and central necrosis of the liver. They observed not only a drop in the fibrinogen following the anesthesia, but a restoration to the normal level after the liver had regenerated some of its lost tissue. Meek[13] found that there was no regeneration of fibrino-

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gen after defibrination in animals in which an Eck fistula had been produced and both portal vein and hepatic artery had been ligated. On the contrary, the fibrinogen which remained in the blood rapidly disappeared. Recent clinical observations also point to the hepatic origin of fibrinogen. Isaac-Krieger and Hiege [14] and McLester and Davidson [15] observed a decrease in the fibrinogen in diseases of the liver, which involve destruction of the parenchyma, including acute yellow atrophy, carcinoma and tuberculosis of the liver.

Observations indicating the rôle of the liver were also made recently by Schultz, Hall and Baker [16] in connection with experimental necrosis and repair of the liver. They produced extensive infarct-like lesions in the liver of dogs by injecting small doses of chloroform into the portal system and followed the repair of these lesions. A marked prolongation in the clotting time of the blood was noted in many of the animals a day or two after the chloroform was administered. Indeed, some of the animals died of hemorrhage despite the precautions which were taken during the operations to ligate all bleeders. The peritoneal cavity usually contained large quantities of unclotted blood. There were also signs of considerable external hemorrhage. In the later stages of repair, three or four weeks after the lesions were induced, and when hyperplasia and hypertrophy of portions of the liver were usually marked the opposite was true. The blood now clotted with extraordinary rapidity, and clung to the autopsy surgeon's hands in long shreds. The studies reported in this paper are the outgrowth of the interest which these observations stimulated.

I. *Method of Determining the Fibrin.* Quantitative methods for the determination of blood fibrin have been reviewed by Robertson,[17] Gram,[18] Foster and Whipple,[19] Howe [20] and especially by Starlinger.[21] It is agreed that the older methods are unsatisfactory. Recent methods, which compare favorably with each other in accuracy, are those of Cullen and Van Slyke,[22] Gram,[18] Foster and Whipple [19] and Wu.[23] They are all alike in that the clot is obtained from the citrated or oxalated plasma by the addition of calcium chloride, but differ in the final steps. Wu determined the fibrin by colorimetric procedure. Cullen and Van Slyke determined it indirectly by the Kjeldahl method, calculating the amount of fibrin from the amount of N recovered. Gram washed the precipitated fibrin with distilled

water, alcohol and ether, dried and weighed it directly. Foster and Whipple dried the fibrin to a constant weight in an oven at 110° C., burned it and weighed the ash, and from the difference calculated the quantity of fibrin. The method of Foster and Whipple is probably on the whole the most satisfactory method, and more fully stated is as follows:

Approximately 9 c.c. of blood are delivered into a graduated centrifuge tube containing 1 c.c. of a 1 per cent sodium oxalate solution. The samples are then centrifuged for 30 minutes to bring down the cells. The cells and plasma are read to tenths of a cubic centimeter. Exactly 2 c.c. of the oxalated plasma are transferred to a "tumbler" containing 40 c.c. of 0.8 per cent NaCl and 2 c.c. of 2.5 per cent $CaCl_2$. This is allowed to stand at room temperature for 2 hours to flocculate the fibrin. The fibrin is freed from its saline bath by gentle manipulation and pressure with a glass rod and is then transferred to a crucible and dried at 110° C. for 3 to 10 hours, or until a constant weight is obtained. The protein is burned, the ash weighed, and from the difference in weight the amount of fibrin is calculated in terms of milligrams per 100 c.c. of plasma and of blood.

This method with two modifications was employed in all of the studies reported below. The first modification is in the amount of sodium oxalate used as an anticoagulant and the second is in the manipulation of the coagulum after recalcification.

In commenting on the amount of sodium oxalate which they employed, Foster and Whipple make the following statements: "The use of 1 c.c. of a 1.0 per cent solution with 9 c.c. of blood is close to the limit of safety. The addition of a little more blood to this mixture will often start blood coagulation." Preliminary studies soon satisfied us that this amount of sodium oxalate is altogether too close to the limit of safety for uniformly accurate results. We finally decided to use 1 c.c. of a 2 per cent solution to 9 c.c. of blood, and all of the figures given below are based upon this amount. By computation a 1.5 per cent solution of sodium oxalate is approximately isotonic with blood. A 2 per cent solution is therefore slightly hypertonic. However, when added in amounts of 1 c.c. to 9 c.c. of blood this degree of hypertonicity is not sufficient to influence appreciably the cell hematocrit readings.

To recover the fibrin from the oxalated plasma we proceed as follows: The "clotting solution," containing 0.8 per cent sodium

chloride and 0.125 per cent calcium chloride, is made up in bulk. It is siphoned off in 40 c.c. quantities into 50 c.c. centrifuge tubes, to which 2 c.c. of the oxalated plasma are added. Flocculation of the fibrin is secured in 30 minutes at 37° C., or within 1 to 2 hours at room temperature. The coagulum is manipulated as suggested by Foster and Whipple, but instead of lifting the water logged mass out of the tube, and drying it for 3 to 10 hours, it has been found possible to bring the coagulum down to a relatively firm, button-like mass by 5 minutes' centrifugation at high speed. This contains much less water and dries to constant weight in 1 hour at 110° C.

All determinations were made on duplicate samples of blood; that is, instead of making duplicate determinations on one sample, as was practised by Foster and Whipple, determinations were made on two samples of blood. This, we believe, is more in accord with acceptable analytical methods, though more blood is required.

The fibrin values in this paper are expressed in mgm. per 100 c.c. of blood, and were calculated by means of Gram's formula (24), which is given below (letters are ours):

$$\frac{(B - C) \times F \times 100}{(B - O) \times 2} = \text{mgm. fibrin in 100 c.c. of blood.}$$

In which,

B = total oxalated blood
C = total volume of cells
$(B - C)$ = total oxalated plasma
F = fibrin in 2 c.c. oxalated plasma
O = volume of oxalate solution

Using 1 c.c. of sodium oxalate solution this may be reduced to

$$\frac{(B - C) \times F \times 50}{B - 1} = \text{mgm. fibrin in 100 c.c. of blood.}$$

With few exceptions the blood samples were all obtained by heart puncture according to the method described by Schultz.[24] Samples of venous blood, the fibrin content of which was compared with that in arterial blood, were obtained from the jugular vein. Most of the animals were bled while under ether anesthesia. A careful study on several animals showed that ether anesthesia does not appreciably influence the amount of fibrin. Essentially the same fibrin values were obtained whether the animals were bled under anesthesia or

without anesthesia. Neither did prolonged ether anesthesia seem to exert any influence.

Dogs were used in all of the experiments. They were carefully selected as to health and vigor, and separated from the stock animals. They were kept in a large room which was supplied with additional heat during the cold nights. They were kept well bedded with shavings, and were carefully watched for signs of distemper. Small box kennels were provided for the animals following operations, where they were kept until they were able to hold their own among the other dogs.

The first group of animals on which experiments were conducted were kept on a diet of table scraps, but the majority of the animals were fed on Spratt's dog biscuits. Dog biscuits were decided on chiefly because we wanted to keep the animals on food of uniform composition. By analysis [25] these biscuits contain 18.9 per cent protein; 3.7 per cent fat and 56 per cent carbohydrates. The dogs were fed immediately after the samples were taken in the morning, and again about 4 P.M. Determinations were made daily on most of the animals.

II. *Normal Fibrin and Hematocrit Values.* Probably the most complete study of normal fibrin values in dogs has recently been reported by Foster and Whipple.[26] Animals fed on "liberal mixed diet" presented values ranging from 140 mgm. to 248 mgm. per 100 c.c. of blood. The average for the series (13 dogs) was *187 mgm.* per 100 c.c. of blood. Fasting animals gave values ranging from 121 mgm. to 219 mgm. per 100 c.c. of blood. The average for this series (10 dogs) was *167 mgm.* per 100 c.c. of blood. A series of determinations on one animal made at intervals of 3 to 15 days gave values ranging from 130 mgm. to 159 mgm. per 100 c.c. of blood. Diets rich in animal protein produced higher fibrin values, while fasting, fat or a carbohydrate diet tended to decrease the amount of fibrin.

Determinations carried out according to the method described above have given us somewhat higher fibrin values than those obtained by Foster and Whipple. Animals fed on a liberal diet of table scraps gave fibrin values ranging from 174 to 391 mgm. per 100 c.c. of blood (Table 1). The average of 29 determinations on 6 dogs was 255 mgm. per 100 c.c. of blood. The greatest individual variation was 36 per cent. This was in a dog on which fifteen daily

determinations had been made. The average individual variation was 15 per cent. This however includes animals on which only two to four determinations had been made. While the variation for the twenty-nine determinations was large (124 per cent), the mean fibrin for the individual dogs varied only 47 per cent. The average

TABLE I

Normal Fibrin and Hematocrit Values; Dogs on a Liberal Diet of Table Scraps

Dog No.	Number determinations	Fibrin Values				Hematocrit	
		Highest mgm.	Lowest mgm.	Mean mgm.	Per cent variation	Mean	Per cent variation
15.....	15	273	174	214	36.2	55.1	14.6
17.....	2	244	243	243	0.4	45.8	2.1
20.....	3	262	235	248	11.5	59.2	5.8
23.....	3	298	252	279	15.3	49.0	9.2
24.....	4	391	322	343	17.6	52.1	8.1
26.....	2	337	299	318	11.1	57.4	0.8

hematocrit reading was 54.2 per cent, and the average individual variation 6.7 per cent.

Still higher values were obtained on dogs fed on dog biscuits. The average of ninety-three determinations on 22 dogs was 308 mgm. per 100 c.c. of blood, or 21 per cent higher than the average for the dogs on a diet of table scraps. The fibrin values ranged from 200 to 441 mgm. per 100 c.c. of blood (Table 2). The highest average for individual animals was 385 mgm. while the lowest was 222 mgm. The former is 25 per cent above the average for the entire series, while the latter is 27 per cent below the general average. The averages of the remaining twenty animals varied less than 15 per cent from the general average. The variation in individual animals was considerably less than between different dogs. The average variation for the 22 dogs was 18.2 per cent. Five dogs gave individual variations of less than 10 per cent; twelve less than 20 per cent, and seventeen (or 77.5 per cent of the animals) less than 25 per cent. The two animals (Dogs Nos. 30 and 46) on which the largest number of determinations were made presented individual variations of 26 and 31 per cent respectively. The average cell hematocrit readings for the entire series was 53.9 per cent, and the average variation was 9.0 per cent. These figures are almost without exception based upon daily determinations.

In as much as these determinations were all made on arterial blood drawn from the left ventricle, and the determinations by previous workers were made on venous blood, a comparative study was made of the fibrin in arterial and venous blood. Duplicate samples of blood procured from the jugular vein were compared with

TABLE 2

Normal Fibrin and Hematocrit Values; Dogs on a Liberal Diet of Dog Biscuits

Dog No.	Number determinations	Fibrin per 100 c.c. blood				Hematocrit	
		Highest mgm.	Lowest mgm.	Mean mgm.	Per cent variation	Mean	Per cent variation
30	12	438	338	385	26.1	55.7	17.6
34	5	441	323	373	26.7	59.6	11.3
37	4	272	228	260	16.3	57.5	12.0
38	2	278	277	278	0.0	67.0	2.0
44	6	330	252	290	23.6	58.4	12.3
46	11	374	258	315	31.0	55.7	21.2
47	3	385	293	329	23.8	40.0	4.5
48	3	292	239	263	18.1	51.1	0.8
49	3	278	208	234	25.2	40.2	12.3
50	4	328	200	265	35.9	39.9	17.9
51	4	318	216	260	32.1	41.8	24.6
57	8	358	294	319	17.8	53.0	8.9
60	3	293	269	277	8.2	55.3	2.2
63	4	269	222	247	17.4	65.2	10.2
66	2	288	266	277	7.6	68.3	1.0
67	2	293	280	287	4.4	53.1	8.0
68	4	292	273	284	6.5	56.8	12.7
71	3	336	261	290	22.3	48.1	4.9
72	3	385	373	380	3.1	49.6	2.0
73	3	394	332	357	15.7	54.6	8.7
74	2	241	203	222	15.7	56.5	1.8
75	2	371	363	367	2.2	47.2	3.6

duplicate samples obtained in the usual way from the left ventricle. Arterial blood throughout gave higher fibrin values. In the first animal (Table 3) the arterial blood ran from 5.4 to 22.9 per cent higher than venous blood; the average difference being 13.2 per cent. Attention is called to the rise in the fibrin which began on March 15th. This was due to an infection in the neck which followed the third bleeding. The fibrin thereupon rose 98 per cent in the arterial blood and 112 per cent in the venous blood. Attention should also be called to the drop in the cells which resulted from the daily re-

moval of 40 c.c. of blood. Determinations made on two other dogs likewise gave higher fibrin values in arterial blood. In one of the dogs the average difference was 7.3 per cent, and in the other 15.2 per cent. These results are in harmony with the observations which were made by Dastre [27] in 1893, but which seem not to have been confirmed. Dastre claimed that blood taken from the inferior vena cava furnished a noticeably smaller quantity of fibrin than did

TABLE 3

Showing the Difference in the Fibrin Content of Arterial and Venous Blood; Dog No. 60

Date	Weight kgm.	Arterial blood		Venous blood		Difference in favor of arterial blood	
		Fibrin	Cells	Fibrin	Cells	Fibrin	Cells
March		mgm.	%	mgm.	%	mgm.	%
12	19.5	269	56.3	255	55.5	14	5.4
13	19.3	293	55.6	262	53.0	31	11.8
14	19.5	269	53.9	230	54.3	39	16.9
15	18.6	354	52.8	288	54.9	66	22.9
17	18.3	535	51.3	487	53.3	48	9.8
18	17.7	537	48.5	477	49.2	60	12.5

arterial blood. It therefore appears that fibrinogen is rapidly consumed by the tissues and that it plays a manifold rôle in the economy of the body.

III. *Factors Influencing the Quantity of Blood Fibrin.* (A) Influence of liver necrosis. Reference has already been made to the alteration in the coagulability of the blood observed by Schultz, Hall and Baker after the injection of chloroform into the portal system. The studies reported in this paper were initiated primarily by these observations. While the influence of prolonged chloroform anesthesia and of phosphorus poisoning, in which generalized effects cannot be ruled out, had already been determined, there had been no studies made on the blood fibrin following the production of hepatic lesions of this type, in which little of the toxic agent reaches beyond the confines of the liver. If administered slowly one is rarely able to detect chloroform in the animal's breath. The effects on the liver, however, become manifest immediately. It becomes strikingly mottled, due undoubtedly to localized circulatory disturbances, though the action on the liver is toxic as well as me-

chanical. The lesions which appear are firm, raised and infarct-like, and range from about one millimeter to several centimeters in diameter. The amount of liver destruction is in a measure proportional to the amount of chloroform injected. One tenth of a cubic centimeter per kilogram of animal weight will, if injected slowly, destroy about a third of the parenchyma. If this dose is doubled, almost complete necrosis of the liver may result. Animals usually do not survive the larger doses.

In general, the decline in the fibrinogen was not so marked as we anticipated from the observations referred to above. The injection of 0.1 c.c. of chloroform per kilo of animal weight caused a drop of

TABLE 4

Showing the effect of 0.2 c.c. of chloroform per kilo injected into the portal system; dog No. 50

Date	Weight	Fibrin	Hematocrit	Remarks
Feb. 11	6.1	200	44.1	Condition normal
" 12	5.7	220	40.8	" "
" 14	6.1	323	38.4	" "
" 15	6.3	328	36.2	" "
" 15 (10.45 A.M.)	....	....	....	Injected 1.2 c.c. $CHCl_3$
" 15 (3.45 P.M.)	....	362	31.8	Very sick
" 16 (8.00 A.M.)	....	22	24.8	" " Died soon after Almost complete necrosis of liver

from 30 to 50 per cent in 6 to 24 hours. By the 48th hour the fibrin generally returned to its previous level, where it sometimes remained for several days, when it rose distinctly above normal. This we attribute to the laparotomy wound, which we found difficult to keep free of infection. Eventually the fibrin returned to its normal level. Small doses of chloroform caused an immediate rise in the fibrinogen. Slight damage to the liver therefore stimulates fibrin production, as does tissue damage in other regions. Considerable damage to the liver seems to be necessary to bring the fibrin to a low level. Table 4 shows the results obtained on an animal injected with 0.2 c.c. of chloroform per kilo. The fibrin dropped from 328 mgm. to 22 mgm. in twenty-two hours. The liver showed almost complete necrosis. It is interesting that in this animal there was an initial rise in the fibrin at the end of six hours, in which time there is usually a well defined drop. We cannot account for this.

Three animals given 0.3, 0.4, and 0.5 c.c. of chloroform per kilo showed a decline in the fibrin within two hours. One showed a drop of 9.6 per cent at the end of the first hour; the second, a drop of 31 per cent at the end of one and a half hours; and the third, to our surprise, showed a rise of 38 per cent at the end of twenty minutes, which was then followed by a rapid decline. All of the animals died within three hours. In all of the animals the fibrin immediately after death was higher than in the previous determination, while the hematocrit reading was a little lower. We have made similar observations on other animals immediately after death.

It should be borne in mind that all of the results in this series of experiments were influenced to some extent by the laparotomy wound, which tends to stimulate fibrinogen production. This we demonstrated on three healthy dogs by subjecting them to the usual operation. The fibrin rose immediately in all of the animals. One of the animals showed a rise of 22.5 per cent in 24 hours; another a rise of 24.6 per cent, and the third, 48.9 per cent. The wound in each case appeared absolutely clean, having been covered with iodinized collodion at the time of the operation. The fibrin continued high for about four days, and then returned to normal. Wound infection always produces a marked increase in the blood fibrin.

The influence of prolonged chloroform anesthesia on the quantity of blood fibrin has been worked out by Whipple and Hurwitz [12] and by Foster and Whipple.[28] They observed a rapid decline in the fibrinogen following a two to three hour period of anesthesia, a gradual restoration of the fibrinogen to its normal level and finally a distinct rise above the normal as the parenchyma was restored. Experiments on three animals have yielded similar results in our hands. The animals were kept under chloroform continuously for three hours. Two of the animals died within 24 hours. One of these presented a drop in fibrin of 38.3 per cent in 15 hours, and the other a drop of 69.0 per cent in 21 hours. Both showed marked central necrosis of the liver. The third animal (Dog No. 57) survived and yielded some interesting figures (Table 5). The fibrin of this animal dropped from an average level of 318 mgm. per 100 c.c. of blood to 28 mgm. in 45 hours, a drop of over 90 per cent. It is interesting that only two days were required for the fibrin to return to its normal level. Regeneration of the parenchyma is far from complete in

this time, so that we must attribute the rapid restoration of the fibrinogen to greater activity of the remaining liver cells. This also explains the rise above normal which appears soon after.

Interesting results were also obtained when large doses of carbon tetrachloride were given by mouth. Schultz and Marx [29] have shown conclusively that carbon tetrachloride is decidedly toxic for the

TABLE 5

Showing the effect of prolonged chloroform anesthesia; dog No. 57

Date	Weight	Fibrin	Hemato-crit	Remarks
	kgm.	mgm.	%	
March 8.....	20.4	335	51.4	Bled without anesthesia
10.....	20.2	312	53.6	" " "
11.....	20.2	304	55.8	" " "
12.....	20.2	300	56.2	Bled under ether anesthesia
13.....	19.7	335	51.8	" " " "
14.....	19.5	358	51.2	" " " "
15.....	19.5	294	51.3	" " " "
17.....	19.5	312	52.2	" " " "
18.....	19.9	....	....	3 hrs. anesthesia (8.45 to 11.45 A.M.)
18.....	19.9	323	55.3	4 hrs. after anesthesia
19.....	18.8	260	63.8	20 " " " Sick
20.....	18.3	28	56.0	45 " " " Very sick
21.....	18.3	197	51.3	74 " " " Sick
22.....	18.1	291	48.9	94 " " " Better
24.....	18.7	357	46.3	Behavior quite normal
27.....	18.2	382	47.1	" " "
31.....	18.6	469	47.3	Active
April 3.....	19.4	291	45.1	"
5.....	19.7	266	49.1	"
7.....	19.3	272	45.7	"

liver, having an action comparable to that of chloroform. Doses as small as 0.05 c.c. per kilo of animal weight when injected into the duodenum produced in some animals outspoken fatty degeneration. Larger doses produced a well marked central necrosis. Similar doses given by mouth produced inconstant results.

Five normal animals were given 4 c.c. of carbon tetrachloride per kilo of body weight by means of a stomach tube, after a series of determinations had been made to determine their normal fibrin level, and the following results were obtained. Two of the dogs showed no drop whatever; two showed a drop of 30 per cent and 48

per cent respectively in 44 hours, at which time they were killed for histological study. The most striking results were obtained on the fifth animal (Dog No. 74). The blood fibrin in this animal dropped in twenty-four hours from its normal level of about 203 mgm. per 100 c.c. of blood to 88 mgm. In fifty-two hours, shortly before the animal was killed, the fibrin had dropped to practically nothing. The trace which presented itself when the plasma was recalcified could not be recovered for gravimetric determination.

The importance of these observations becomes manifest when the histological findings are described. The two animals which showed

TABLE 6

Showing fibrin values during infection

Dog No.	Number determinations	Fibrin per 100 c.c. blood			Condition
		Highest mgm.	Lowest mgm.	Mean mgm.	
16.....	19	709	357	470	Distemper and wound infection
17.....	4	512	402	455	Wound infection
19.....	7	659	324	444	Distemper
22.....	10	474	343	411	Granuloma on nose
23.....	7	750	416	507	Distemper and wound infection
27.....	12	538	411	459	" " " "
33.....	6	695	494	596	"
42.....	3	676	438	571	"
60.....	3	537	354	475	Cellulitis, neck
78.....	2	686	678	682	Distemper
80.....	5	480	396	437	"
81.....	2	820	806	813	"

no drop in fibrin showed no lesions whatever in the liver. The two animals which showed only a moderate drop in fibrin showed a fatty degeneration of the liver, with beginning central necrosis. The fifth animal, in which the fibrinogen had almost disappeared, showed almost complete necrosis of the liver. In some areas the lobules were completely destroyed, while in other areas only islets of liver cells were found surrounding the portal units.

(B) Influence of acute infections and suppuration. High fibrin values have been reported in acute infections and suppurative processes by Mathews,[3] Müller,[6] Morawitz and Rehn,[7] Foster and Whipple,[28] McLester [30] and others. McLester and Davidson [15] recently reported that they obtained low fibrin values in typhoid

fever, but high values in pneumonia and septic states. High fibrin values have been found not only in acute infections, or following the injection of bacterial products, but also following the production of sterile abscesses and other types of tissue injury. The termination of an acute infection, as the crisis in pneumonia, or the drainage of a sterile abscess, is followed by a rapid decline in the fibrinogen. Table 6 gives some values obtained on dogs with distemper, wound and other infections.

Summary

Normal fibrin values were obtained on 28 dogs, representing a total of 122 determinations. The average for animals on a diet of table scraps was 255 mgm. per 100 c.c. of blood, while the average for animals fed on dog biscuits was 308 mgm. per 100 c.c. of blood. Arterial blood yielded slightly higher fibrin values than venous blood.

Liver necrosis produced a decrease in the fibrinogen. Except when the damage to the liver was severe, the fibrinogen returned to its normal level within forty-eight hours. Chloroform injected into the portal system produced an immediate decline in the fibrinogen ranging from 30 to 90 per cent, depending on the amount injected and the length of time the animal lived. A similar decline followed prolonged chloroform anesthesia and carbon tetrachloride poisoning. One of the animals poisoned with carbon tetrachloride showed a complete disappearance of the fibrinogen. The liver of this animal had undergone almost complete necrosis.

Mild liver injury produced an immediate rise in the fibrinogen, as in the case of tissue injury elsewhere. The fibrinogen also rose immediately following a clean laparotomy. Acute infections produced the highest elevations. Ether anesthesia produced no noticeable effect.

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THE RELATION OF CHRONIC POISONING WITH COPPER TO HEMOCHROMATOSIS *

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Introduction. In a previous paper [1] two apparently unrelated subjects were presented, a histological study of the lesion of hemochromatosis in man and the results obtained from chronic poisoning with acetate of copper fed to rabbits. The present paper gives a summary of the former work, records certain aspects of the study of ten cases of hemochromatosis which came to autopsy at the Boston City Hospital within one year since then, and presents further experimental work with copper poisoning.

The main points of the first paper can be stated very briefly. In hemochromatosis a yellow pigment, hemofuscin, derived from hemoglobin, is deposited in the endothelium lining the sinusoids, and in the parenchymatous cells of the liver. In the course of time it is changed to hemosiderin. The transformation is very slow and requires at least months and probably years. When the parenchymatous cells are filled with pigment beyond a certain degree they undergo necrosis and the pigment is taken up by endothelial leukocytes which often collect in numbers, especially in the periportal connective tissue. Following necrosis, regeneration of liver cells occurs diffusely and in islands. The new cells in time become pigmented with hemofuscin which later changes to hemosiderin. Owing to the necrosis and disappearance of the liver cells, the stroma in places is relatively increased in amount by coalescence, resulting in sclerosis. In the foci where the liver cells regenerate, new stroma is formed as in a tumor. In this way the connective tissue in the liver is gradually increased in actual amount. It is possible to find all stages of the process terminating in pigment cirrhosis present in a section from a liver in which the changes are active.

Hemofuscin is also deposited later and more slowly in the fibroblasts of the stroma, especially around the larger blood vessels, in the smooth muscle cells of the arteries and veins and in the bile

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duct epithelium. In all these latter cells the hemofuscin is changed very slowly or not at all into hemosiderin.

Pigment appears early in the kidney, chiefly in the epithelium of some of the tubules, but never extensively. The cells killed by the deposit desquamate, are carried away by the secretion and replaced by regeneration. Neither accumulation of pigment nor sclerosis occurs.

After the liver cells have taken up about all the pigment they can hold it begins to be deposited in the other organs and tissues, especially in the pancreas, the cortex of the adrenal glands, the lymph nodes in the upper part of the abdomen, the heart, thyroid, skin of the extremities, mucosa of the stomach, etc. In the pancreas, necrosis, regeneration and sclerosis occur as in the liver; the physiological effect is to cause diabetes mellitus. Destruction of the cortex of the adrenal glands may lead to increase of the normal pigment (melanin) in the skin and other tissues, as in Addison's disease. Pigmentation of the heart may result in necrosis of muscle fibers and the formation of patches of sclerosis.

Chronic poisoning of rabbits with acetate of copper in small doses (100 mgm. or less daily on food) leads to the gradual deposit of a yellow pigment, hemofuscin, in the endothelium and parenchymatous cells of the liver. In three months' time necrosis and regeneration are active and many endothelial leukocytes are present filled with the pigment granules. In six to twelve months the animals die from sclerosis (cirrhosis) of the liver accompanied with jaundice. The hemofuscin shows slight or no change to hemosiderin.

The incidence of hemochromatosis. Hemochromatosis is usually considered a rare disease. During one year, March 1, 1922, to March 1, 1923, we had at the Boston City Hospital 288 postmortem examinations on adults. Ten of the bodies, 3.4 per cent, showed well-marked pigment cirrhosis with grossly evident deposit of hemosiderin in the upper abdominal lymph nodes and in the pancreas. Of the ten cases, four showed pigmentation of the skin, four had jaundice, two ascites, and one primary liver cell carcinoma. Only one, the last, was suspected of hemochromatosis during life and proved positive by excising a piece of skin and performing the iron reaction. All the other cases were accidental findings at the autopsy table. None had gone far enough for sugar to be demonstrated in

the urine. The patients did not die from hemochromatosis but from intercurrent complications such as lobar pneumonia, meningitis, etc.

Besides these ten cases of well-marked hemochromatosis there were three with a slight to moderate degree of pigment cirrhosis and nine showing hematogenous pigments in the liver, pancreas, heart and kidneys. They represented earlier stages or milder degrees of the process. The diagnosis in each instance was based on the presence of the two pigments characteristic of the disease, namely, hemofuscin, which was found practically only in the stroma, especially around the larger blood vessels, and hemosiderin, which occurred chiefly in the parenchymatous cells. In pernicious anemia much hemosiderin is often present in the liver and other organs, but hemofuscin does not occur except in an occasional case complicated with a slight degree of hemochromatosis.

In the year following this run of ten cases of well-developed hemochromatosis, under identical conditions and with interest unabated, no well-marked case came to autopsy.

Regeneration. One case, A23.48, in this series is of much interest because the process is active and in consequence the liver shows many small foci of liver cells, up to 2 mm. in diameter, at various time periods following regeneration. The cells in some foci contain no pigment, in others only hemofuscin, while in still others hemofuscin and hemosiderin occur in varying combination as the first pigment is gradually being transformed into the second.

In the pancreas a similar deposition of hemofuscin in regenerated acinar cells and its transformation into hemosiderin can be seen.

In two cases occurring in earlier years (A17.8 and A19.24), the liver showed similar islands of regeneration with various stages of the process of deposit of hemofuscin and transformation to hemosiderin.

In the liver of another (A22.164) of the series of ten cases the foci of regeneration are much larger, measuring many centimeters in extent, and resemble somewhat the picture presented by a liver of acute yellow atrophy during the stage of regeneration. The large amount of hemosiderin present in the heart and kidneys in this instance suggests a much more active process than is usually found.

These four cases with foci of regeneration enable one to follow clearly the deposition of hemofuscin and its transformation into

hemosiderin but the time required for the change cannot be determined.

Chemical examination of organs for copper. The chief interest in this unusual series of cases lay in the opportunity it afforded of finding out, if possible, if chronic poisoning with copper could be connected with them in any way. Previous experimental work had failed to demonstrate copper in the hemofuscin even in the livers of rabbits poisoned with this metal.

Chemical examination of the liver and in some instances of certain other organs gave the following results. The first two examinations were made by a cruder method which would not show minute amounts.

	Tissue	Amount	Copper
A22.64	Liver (most of organ)	....	0. mgm.
A22.67	Liver (most of organ)	....	0. "
A22.164	Liver and other organs	1086 gm.	3.87 "
A22.286	Liver and kidney	1045 gm.	20. "
U22.11	Liver and other organs	952 gm.	3.35 "
A23.48	Liver	588 gm.	0.87 "
	Bone	160 gm.	0.50 "

Examination of the livers from several controls yielded somewhat similar figures.

	Tissue	Amount	Copper
A22.274	Liver	1095 gm.	15.5 mgm.
A22.275	Liver (Addison's disease)	980 gm.	4.5 "
A22.289	Liver	1930 gm.	1.5 "
M.L.C.	Liver (Alcoholic cirrhosis) . . .	2450 gm.	50. "

These results seemed to demonstrate quite conclusively that this line of investigation would not be a profitable one and it was therefore discontinued.

The cause of hemochromatosis. A careful study of the clinical histories [2] of the series of ten cases and also of the others occurring in previous years was made in order to find out what light they might throw on the cause of hemochromatosis. Unfortunately the records were found often inadequate or even wholly wanting owing in part at least to some of the patients having been brought in unconscious or in a dying condition without family or friends appearing.

Only two factors seemed to have any definite bearing on the pro-

duction of the disease, excessive indulgence in alcohol and contact with copper, chiefly through occupation. Some individuals had been exposed to both.

Two of the ten cases, A22.28 and U22.4, both females, 65 and 60 years of age, have entirely negative histories, except that one was stated to be "a good liver." With them belong two living cases, males who have cirrhosis of the liver, diabetes and pigmentation of the skin. Repeated questioning has failed to reveal any source of poisoning by copper.

Alcohol. Excessive indulgence in alcohol has frequently been noted as a possible etiological factor in the production of hemochromatosis. In this series of ten cases at least two, A22.164, male, 49 years, ship-builder and A22.315, male, 56 years, painter, had both used alcohol to excess for many years.

In the twenty-five years preceding the occurrence of these ten cases there had been seven cases of whom three, U02.39, female, 31 years, A19.24, male, 44 years, and A21.25, male, 55 years, are all stated to have drunk alcohol to excess. Two others, A15.117, male, 50 years, importer of Italian liquors, and A05.21, male, 84 years, longshoreman, are noted to have been alcoholic although one of them is said not to have been a drunkard. Still another, A17.8, male, 42 years, teamster, with typical and marked lesions of hemochromatosis complicated with slight alcoholic cirrhosis, had also drunk to excess.

One case of hemochromatosis, M1010, male, 51 years, occurring at the Peter Bent Brigham Hospital this past year is of much interest in this connection. The patient came in for treatment of hemorrhoids. He was found to have an enlarged liver, sugar in his urine and marked pigmentation of the face, neck and extremities. Examination of an excised piece of skin showed an abundance of yellow pigment in the corium, around the sweat glands and in the underlying fat tissue. The reactions for iron were strongly positive, indicating hemosiderin.

The man had been a bar-keeper for four years previous to National Prohibition and had consumed about one pint of whiskey and four to five glasses of beer daily. For the past six years, since the enforcement of prohibition, he had been a boot-legger and had also run a private copper still of his own, making about two gallons of corn

whiskey at a time in a tin-lined copper still, but with the coils in the condenser made of pure unlined copper.

Copper in alcoholic beverages. The occasional occurrence of lead in liquors, especially of the "moonshine" variety, in sufficient quantity to cause symptoms of acute lead poisoning suggested that copper also might be found. A limited number of chemical examinations gave the following results.

In six different wines the highest amount of copper to the liter was 1.68 mgm., in four fortified wines 0.89 mgm. In seven out of eight distilled liquors, chiefly of the variety known as "hooch," the amount in a liter varied from a trace to 10 mgm., but in the eighth it reached 185 mgm. A sample of "home-brew" made and drunk by a patient with cirrhosis of the liver and ascites gave 25.5 mgm. of copper to the liter. In the liquor removed from a still seized by the Boston police it amounted to 1250 mgm. to the liter (this would equal 4.95 grams of copper sulphate). This sample indicates the action of the acids in the ingredients to be distilled on a copper container. The copper salt would not, of course, distil over, but the volatile acids (chiefly acetic and citric) passing over with the alcohol during distillation would act in the same way on the copper coil or worm of the condenser and lead to the presence of more or less copper in the resultant liquor. This is evidently the way in which copper gains entrance to distilled liquors.

It was found that the ferrocyanide of potassium test for copper could be used perfectly readily on liquors. The only effect of the alcohol is to cause the precipitate of cupric ferrocyanide to occur in a flocculent form and to settle readily. Amounts as low as 10 mgm. to the liter were recognizable. By concentration through evaporation smaller amounts can be detected. The method furnishes a simple way of spotting liquors containing considerable copper and gives some idea of the amount. By the use of this method the Department of Public Health of Massachusetts was able to detect copper in nine out of eighty-four samples of "hooch" examined. The amounts present in five of the samples ran approximately 6, 10, 20, 25 and 26 mgm. of copper to the liter. The other four varied from a trace to 3 mgm. For exact determination quantitative methods must be used.

Two of the fortified wines contained lead to the amount of 53.0 and 74.1 mgm. to the liter.

The frequent association of alcoholic hyalin with pigment cirrhosis suggests that the cause of these two different types of lesions may often be contained in the same beverage.

Occupation. An attempt was made to find out if exposure to copper in consequence of occupation had any bearing on the production of hemochromatosis. The results obtained are interesting and suggestive but not definite and conclusive. Four of the ten cases had come in contact with copper for many years, A23.48, male, 63 years, an alcoholic, had worked at a lathe for two years milling and planing brass and then had continued for thirty-six years working on high grade steel in the same large dusty room. A22.67, male, 55 years, forged metals for twenty-five years in a dusty railroad shop where copper was handled. M941, male, 55 years, an alcoholic, polished pipe wrenches for thirteen years in the same place where brass pipes were milled and worked. A23.37, male, 46 years, fixed cables and telegraph wires as a lineman for twenty-three years.

One other case which came to postmortem examination at another hospital has a much clearer history of exposure to copper dust without alcohol playing any part in the etiology. U22.11, male, 41 years, had worked for fourteen years in a shop "milling and turning copper and brass" and then for two years had served as a plumber's clerk.

Three of our hospital cases dying of other diseases are of interest in this connection, although they had no pigment cirrhosis. A24.110, male, 49 years, worked in a brass foundry for eight years. A24.124, male, 60 years, was a copper worker since the age of fifteen. A23.19, male, 52 years, came to this country sixteen years ago. For six years he worked in a brass foundry, then, owing to brass (zinc) colic, he worked for the next ten years in an iron foundry. All three cases had the two characteristic pigments, hemofuscin and hemosiderin, in varying amounts in the liver, pancreas, heart and kidneys, chiefly in the fibroblasts of the stroma around the larger blood vessels. The first two had slight pigmentation of the extremities and skin excised during life gave a moderate but typical iron reaction.

In view of the apparent relation between exposure to copper dust and hemochromatosis suggested by this series of cases it was de-

termined to try to find out by animal experimentation what the pathological effect of swallowing or inhaling copper powder would be.

Copper dust. Copper dust inhaled may reach the lungs, or, in part at least, be swallowed with pharyngeal secretion or with sputum coughed up from the trachea. In the stomach the action of the hydrochloric acid theoretically at least should lead to the formation of a copper salt although text books on chemistry state that dilute hydrochloric acid has no effect on pure copper. Absorption of a copper salt should in time, if in sufficient quantity, result in pigment cirrhosis as in animals.

Fine copper powder affords a very simple and quick way of testing the effect of acids and alkalies on the metal, owing to the large amount of surface exposed. It was found that by shaking the powder vigorously in water or other fluids for ten to fifteen minutes a complete temporary suspension could be obtained with most of them. After standing a few minutes the copper settles to the bottom of the test tube and the supernatant fluid can be filtered. A series of experiments were tried.

The filtrate from a suspension of 200 mgm. of fine copper powder in 10 c.c. of distilled water gave no reaction with a two per cent solution of ferrocyanide of potassium. The filtrate from a similar suspension in a one per cent solution of nitric acid was pale greenish and gave an immediate heavy reddish brown precipitate with the same reagent. With hydrochloric acid the filtrate was colorless and gave a slowly forming whitish precipitate with the ferrocyanide. If, however, a minute amount of nitric acid or permanganate of potassium was added to the filtrate, to oxidize the copper salt present, or the filtrate was simply allowed to stand for twenty-four to forty-eight hours a strong characteristic precipitate occurred on adding the ferrocyanide. The filtrate from a suspension in blood serum gave a well marked reaction. These experiments demonstrate that copper powder dissolves to more or less extent in various acid and alkaline solutions.

Animal experiments. Five rabbits were given copper powder (100 to 200 mgm. to each animal) on their food daily. In two months' time two of the five died with pigmentation and beginning necrosis

of liver cells. The pigment occurred as yellow granules in liver cells and in endothelial leukocytes, most abundantly at the peripheries of the lobules, and in sections of fixed tissue stained slowly but deeply with basic aniline dyes (fuchsin and methylene blue), a characteristic of hemofuscin. Similar granules were found in the endothelium lining the sinusoids of the liver, the capillaries of the heart, in some of the renal cells and in fibroblasts around the larger blood vessels in the liver and kidneys. Examination of the feces in the large intestine showed only the coarser granules of copper to be present.

To test the action of the alkaline fluids of the body on copper, so as to be able to form an idea of what would happen to copper dust carried into the lungs, 100 mgm. of copper powder suspended in a two per cent solution of gelatine were injected subcutaneously in a rabbit, which was killed at the end of six days. There was extensive necrosis and inflammatory reaction at the site of injection. The adjoining skeletal muscle fibers were necrotic, often calcified and were surrounded by foreign body giant cells. The copper particles had all been dissolved except the coarser ones and they were surrounded by yellowish crystalline material. Hemofuscin granules in large numbers were present in the liver cells and endothelium, in many of the renal cells and abundantly in the capillary endothelium of the heart. They occurred in smaller numbers in fibroblasts around the larger blood vessels of the liver and kidney while the fibroblasts and endothelium of the bone marrow were filled with them.

A rabbit injected intravenously with 50 mgm. of copper powder in a gelatine suspension and with 100 mgm. on the next day, died in thirty-six hours. Delicate hemofuscin granules were already present in the liver cells, especially at the peripheries of the lobules, and to a slight extent in fibroblasts around the larger blood vessels.

Twelve milligrams injected intratracheally into a rabbit, which was killed at the end of ten days, caused necrosis of lung tissue with acute inflammatory reaction. The liver contained considerable hemofuscin in the parenchymatous cells, in the endothelium of the sinusoids and to a slight extent in the fibroblasts.

These experiments demonstrate clearly that copper powder obtaining entrance to the body through the gastrointestinal or respiratory tract is readily dissolved and absorbed and causes the deposi-

tion of a yellow pigment, hemofuscin, in the liver, heart, kidneys, bone marrow and probably other organs. The same is true of copper powder injected intravenously and subcutaneously.

Because the solution of gelatine was found to be slightly acid in reaction some of the experiments were repeated with a suspension of the powder in distilled water; identical results were obtained.

Copper in foods. One of the ten cases, M932, male, 50, worked eighteen years in a cannery cooking fruit in copper kettles. The question naturally arose as to whether he could have acquired chronic poisoning with copper owing to the nature of his occupation.

The presence of copper in foods does not seem to have attracted much attention as yet beyond the recognition of its use to color pickles and canned peas and beans. It is a problem for the chemist to attack.

Tests with several organic acids in one per cent solutions showed that citric, acetic, lactic, tartaric and tannic acids readily attack powdered copper. The most powerful is citric which equals nitric, the recognized solvent of copper. Acetic comes next and the others range below it. Sweet cider (malic acid?) has a similar effect. Because apple butter is regularly made in copper kettles, sweet cider was allowed to stand in a clean, bright copper kettle for twenty-four hours. A well marked reaction was obtained with ferrocyanide of potassium while the control was negative. It seems probable that acid fruits cooked in copper kettles are to be regarded with suspicion, and careful chemical examination should be made of those which, like apple butter, grape jelly and tomatoes, are most likely to be contaminated by cooking in copper kettles.

Copper kettles, coffee pots, water pipes, frying pans, etc. Three of the ten cases studied had used copper kettles for boiling water for cooking purposes. One of them had also worked at a copper occupation and a second had imbibed alcohol to excess. The third, however, had led an outdoor life and the use of alcohol was denied.

The filtrate from tap water boiled with copper powder in it gives no precipitate with ferrocyanide of potassium but there is other evidence of the action of tap water.

The hot and also in some instances cold, water pipes put into many houses in Brookline and other towns and cities years ago

were of brass. In the course of fifteen to twenty years they became so eroded that a pin often could be thrust through them and they had to be replaced with iron pipes. Evidently substances in the water had gradually dissolved both the copper and the zinc.

Copper and brass teapots are sometimes used; copper coffee pots are usually lined with silver, nickel or tin, but the lining is often removed through industrious scouring by cooks; both utensils, owing to the action on them of the tannic acid contained in tea and coffee, represent a possible source of chronic poisoning with copper. The same is even more true of similarly lined cocktail shakers because of the citric and other acids often present in the ingredients; they act on tin as well as on the copper. Beer requires investigation because one step in the process of making it is to pass the wort to the "copper" (large copper kettle or boiler) where it is boiled with hops.

The popular gas hot water heaters of the present day also may be a source of danger if the water passing through them is used for cooking or drinking purposes.

No positive evidence against this class of copper utensils and pipes has as yet been obtained, but they should all be regarded with a certain amount of suspicion. While the danger of poisoning from these sources is probably very slight it must be borne in mind because the possibility exists.

The filtrate from melted lard shaken with copper powder for fifteen minutes is bright bluish green in color owing to the formation of oleate and possibly other fatty acid salts of copper. Melted palmitic and stearic acids are colored blue in the same way. These experiments illustrate the danger of frying and cooking fatty substances in copper pans and kettles. This source of poisoning must be considered very real.

Incidence of hemofuscin in liver and other organs. The liver, pancreas, heart and kidneys were examined from all autopsies over a period of two and a half years in order to determine the frequency of occurrence of hemofuscin in them. It was found only in adults, of whom the youngest was forty-five and the oldest eighty-three. In 463 adults it occurred 54 times or in 11.6 per cent. It was always associated with hemosiderin although the latter was sometimes present only in a small amount. The hemofuscin was found chiefly

in fibroblasts where the connective tissue was most abundant, especially around the larger blood vessels. It is in this location that hemofuscin is changed most slowly or not at all to hemosiderin and therefore persists more or less indefinitely because of its insolubility. In parenchymatous cells it is gradually changed to hemosiderin, which is slowly dissolved and finally eliminated so that these cells may eventually become free of pigment if no fresh deposit occurs.

Four recent cases of atrophic cirrhosis (A23.109, male, 78; A23.144, male, 54; A24.3, male, 65; and A24.25, male, 74) are of interest in this connection. They are all evidently old cases of hemochromatosis in which pigment deposit has long since ceased so that reparative changes have been going on for years. Most of the hemosiderin has disappeared, but a certain amount along with hemofuscin is still present in the liver, pancreas, heart, kidneys and adrenals.

The significance of these findings is that ingestion of copper (if it is the cause of hemochromatosis) must be fairly common, that the body can handle and eliminate a certain small amount without injury to the organs, but that if the amount exceeds a certain minimum and is taken in over a long period of time, lesions are produced which may eventually cause the signs and symptoms of the disease known as hemochromatosis.

Hemofuscin. The nature of hemofuscin is not easy to ascertain, but it is probably only an intermediate product between hemoglobin and hemosiderin with properties different from either. It is preserved by all fixatives, is insoluble in water and dilute acids and stains deeply with basic aniline dyes while hemoglobin stains with acid dyes and hemosiderin with neither. The transformation of hemofuscin into hemosiderin can be followed in the regenerated foci in the liver of man and in the livers of experimental animals fed or injected with copper powder either by killing a series of them or by removing pieces of the organ surgically at various time intervals.

In some of the lower sea animals copper takes the place of iron in hemoglobin. It also forms a compound, cuprohemol, with hemol, a reduction-product of hemoglobin. In view of these facts and because poisoning with copper brings about the deposition of hemofuscin in the liver it would seem as though this pigment must contain copper. All the evidence obtainable thus far, however, is against

this view. It has not been possible to demonstrate copper in hemofuscin, even in that freshly formed in rabbits' livers, by either the hematoxylin or the triple nitrite (K, Pb, Cu) test.

Chronic poisoning with zinc salts causes a deposit of hemofuscin in the liver just as copper does and the same type of lesion: therefore, copper evidently is not necessary in order to form hemofuscin.

A rabbit injected subcutaneously with 0.75 gm. of copper powder suspended in distilled water died at the end of three days. Besides early but well-marked pigmentation in the liver there was also central necrosis of this organ, indicating the toxicity of the soluble copper salt formed by the dissolving action of the lymph. In addition numerous hemoglobin casts were present in the tubules of the kidneys.

Sheep are extremely sensitive to poisoning with copper and are killed by doses no larger than those effective with rabbits. They die not from the pigmentation of the liver, which is well-marked, but from occlusion of the renal tubules by hemoglobin casts.

This occurrence of hemoglobin casts in the kidneys in these experiments demonstrates destruction of red blood corpuscles and the setting free of a large amount of hemoglobin which evidently is not bound to copper. A slight degree of anemia has often been noted in patients afflicted with hemochromatosis.

In old hemorrhages, such as occur as the result of menstruation in chocolate cysts of the ovary due to uterine implants, where the hemoglobin is slowly transformed into hemosiderin, an intermediate yellow compound is formed which occurs often abundantly in small and large granules in fibroblasts and endothelial leukocytes. These granules stain deeply with basic aniline dyes and in other ways react like hemofuscin. This occurrence would seem to demonstrate beyond question that hemofuscin is only an intermediate product between hemoglobin and hemosiderin. Even better evidence on this point was afforded by injecting the hemoglobin obtained by the ether method from 25 c.c. of rabbit's blood into a medium sized rabbit. In twenty-four hours the liver cells contained numerous coarse granules of hemofuscin and the renal cells many fine ones.

Perhaps all that chronic poisoning with copper does is to cause a slight but persistent destruction of red blood corpuscles with setting free of hemoglobin.

The pigment deposited in the liver and other organs as the result of the action of copper does not at first stain readily with basic fuchsin. Hours instead of minutes are required. The granules in the endothelium lining the sinusoids are the first to stain deeply. Those in the liver cells follow much later. This difference in staining reaction would seem to indicate that the pigment which is first deposited slowly undergoes some transformation before it acquires the properties of hemofuscin.

Time necessary to produce hemochromatosis. Two questions are of interest under this heading. How long a time is required to change hemofuscin to hemosiderin and how many years does it take to produce the disease known as hemochromatosis?

Hemofuscin changes to hemosiderin very slowly in the rabbit: two to three years are required before granules begin to give a good reaction. In the sheep the process is more rapid so that hemosiderin can be demonstrated in less than a year. In a small South American monkey, as already reported,[1] the change was easily evident at the end of five months. The change in man is probably not any more rapid.

To produce a well-marked example of the disease hemochromatosis in all probability requires at least ten years and more likely fifteen to twenty or more. The great majority of cases are from forty to sixty years of age. The youngest listed here was thirty-one, and none younger has been found in the literature. The most definite history in regard to time is that of the man who "worked in a shop milling and turning copper and brass" for fourteen years and then lived two years longer.

Summary

Ten cases of hemochromatosis came to postmortem examination in one year at the Boston City Hospital; none was seen in the following year under identical conditions, hence the occurrence must be regarded as due to coincidence only.

Nine other cases occurring before or since this group of ten were also available for study, making a total of nineteen. Nine had used alcohol steadily for many years, usually to excess. Six were exposed to chronic poisoning with copper owing to their occupation (four in shops for thirteen to thirty-eight years milling and planing brass and

copper or inhaling the dust from these metals, one as lineman for twenty-three years for a telephone company, one in a cannery for eighteen years cooking fruits in copper kettles). One had regularly used a copper kettle for boiling water, three had entirely negative histories.

Chemical examination of the liver and of some of the other organs from several of these cases showed a small amount of copper but no more than was obtained from controls.

The cause of the disease seems to be connected chiefly with two different subjects, with alcohol and with an occupation involving copper.

Analyses of a limited number of distilled liquors gave from a trace to 185 mgm. of copper to the liter, evidently derived from the solvent action of organic acids on the copper worm of the condenser.

The ferrocyanide of potassium test will give a visible reaction with distilled liquors containing from 10 mgm. of copper up. By concentration through evaporation smaller amounts can be recognized.

A man drinking daily a quart of whiskey containing 185 mgm. of copper to the liter will take into his system about a gram of copper a week, which is, proportionately, comparable to the amount required slowly to produce pigment cirrhosis in rabbits and monkeys.

Fine copper powder shaken up in one per cent aqueous solutions of nitric, hydrochloric, acetic, tartaric, lactic and citric acids for fifteen minutes was dissolved to a perceptible degree. Some of the filtrates were colored green and all gave strong characteristic reactions with ferrocyanide of potassium. Citric acid was apparently about as active as nitric; acetic and the other acids ranged below them. The action of "sweet cider" on copper was strong, while lard melted and shaken with copper dust for fifteen minutes was found to be bluish green when filtered, owing to the solvent action of oleic acid. Palmitic and stearic acids were colored blue in the same way.

Fine copper powder fed on food to rabbits was dissolved in the gastrointestinal tract and caused death in two months with marked pigmentation of the liver. Injected intravenously, intratracheally or subcutaneously it was quickly dissolved by the fluids of the body, causing marked local reaction and pigment deposit in the liver, kidneys, bone marrow and some of the other organs and in some instances death in thirty to seventy-two hours.

Chronic poisoning with copper in sheep and acute poisoning in rabbits caused, in addition to pigmentation, blocking of the tubules of the kidneys with hemoglobin casts, indicating destruction of red blood corpuscles.

Hemofuscin, one of the two pigments always present in hemochromatosis, is apparently nothing but an intermediate product between hemoglobin and hemosiderin. It is deposited in the liver and other organs of animals as the result of poisoning both with copper and with zinc, but it is also found in the liver and kidney following intravenous injection of hemoglobin and in the cells of the tissues surrounding old hemorrhages.

The change of hemofuscin to hemosiderin can be followed in the livers of animals poisoned with copper and also in the islands of regenerated cells in the liver of man.

Microscopic examination of the liver, pancreas, kidneys and heart in all adult human beings (463) coming to autopsy during two and one half years showed hemofuscin and hemosiderin present in fifty-four, or 11.6 per cent.

Conclusions

Evidence is steadily accumulating to prove that chronic poisoning with copper is the cause of hemochromatosis. While the fully developed disease is relatively rare, the early stages and the lighter forms are fairly common, but are necessarily unrecognized by the clinician and commonly overlooked by the pathologist.

The sources of the poisoning are (1) distilled liquors contaminated with copper dissolved from the copper worm of the condenser by the action of volatile organic acids (citric, acetic, etc.); (2) occupations involving exposure to copper dust (brass foundries, brass milling, planing and polishing, telephone line-repairing) as the metal is readily dissolved in the juices of the body however it may obtain entrance, and (3) probably also acid foods, jellies, candies, etc., contaminated by copper owing to having been cooked in copper vessels. Under this last heading must be included foods cooked and fried in copper kettles and pans owing to the dissolving action of oleic, palmitic and stearic acids in lard and other fats on the metal.

Experimental work with animals demonstrates that copper inhaled or ingested is dangerous to life although its action as a chronic

poison is exceedingly slow. This is the reason its deleterious effect has been so long overlooked.

Studies of clinical cases show that ordinarily it takes fifteen to twenty-five or more years to produce the symptom complex of the disease known as hemochromatosis.

Now that the danger of poisoning has been pointed out, steps should be taken to prevent copper getting into liquors and foods and to protect workers in occupations involving copper from inhaling or ingesting copper dust.

The reason that copper is so generally found in minute quantities in human organs is not due to its being a normal constituent of the tissues but because man is constantly exposed to taking the metal into his system through foods and drinks contaminated with it. While small amounts cause no harm, because slowly eliminated, larger amounts absorbed during many years are exceedingly dangerous. Susceptibility to poisoning by copper probably plays a part as with lead and arsenic.

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